JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.2024016

Survival Time of *Campylobacter jejuni* in Broiler Crops

Mari Nishii and Masaharu Yasutomi†

Kyoto Prefecture Agriculture Experiment Station, Ayabe-Shi 623-0221, Japan

Lactobacillus spp. inhibit the growth of *Campylobacter* spp. *in vitro*. However, in chicken crops, in which *Lactobacillus* spp. predominate, such inhibition of *Campylobacter* has not been confirmed. In our previous study, feeding paddy rice to broiler chicks increased the residence time of the food, which might enhance the bactericidal activity of the crop. Here, the bactericidal activity against the remaining *Campylobacter* spp. in broiler crops was evaluated. A suspension prepared by mixing *Campylobacter jejuni* and titanium dioxide (TiO_2) was inoculated into the pharynx of 26-day-old broiler chicks fed a paddy rice-based diet. The crop contents were sampled at 20-min intervals. The TiO_2 residual ratio in the crop gradually decreased with time after inoculation, with 57% of the inoculated TiO_2 remaining in the crop 60 min after inoculation. The survival fraction of *C. jejuni* in the crops was 11% at 40 min, only 1% at 60 min, and was undetectable at 80 min. Most of the inoculated *C. jejuni* died in the crop before entering the next segment. These data indicated that bacterial death occurred between 30 min and 40 min after inoculation. The average survival time of *C. jejuni* in the crop was calculated to be 37.1 min. Thus, *C. jejuni* remaining in a chicken crop for more than 40 min died.

Key words: broiler crop, *Campylobacter*, lethal event, retention time, survival fraction, whole-grain paddy rice

J. Poult. Sci., 61: jpsa.2024016, 2024

Introduction

Campylobacter is a leading cause of enteric zoonotic infections[1]. *Campylobacter* infection is a major public health challenge with a complex epidemiology involving widespread animal and environmental reservoirs and multiple risk factors[2,3]. *Campylobacter* has been detected in soil, groundwater, wild animals, and insects[4–7], indicating its transmission through footwear and clothing of farm workers, flies, and small animals.

Domestic poultry (e.g., broilers, layers, turkeys, and ducks) have been identified as natural reservoirs of *Campylobacter*[8,9]. Broiler chicks are colonized by *Campylobacter* primarily in their ceca between 2 and 4 weeks of age[10]. Once some chickens are infected with *Campylobacter*, most broiler flock birds become colonized within a few days and remain infectious until age of slaughter[11–13].

Although broiler chicks are natural reservoirs of *Campylobacter*, accidental ingestion of *Campylobacter* does not always lead to colonization of the lower gastrointestinal tract. Chickens have several natural defense barriers in the upper digestive tract that eliminate pathogens invading the oral cavity[14]. Gastric juice secreted from the proventriculus kills microorganisms by lowering the pH of the gizzard[15]. This natural defense barrier is enhanced by the addition of insoluble dietary fiber to the feed[14]. In our previous report[16], the diluted nutrients and hardness of insoluble fiber equivalent to 20% of paddy rice accelerated the grinding activity of the gizzard, which homogenized the pH value in the gizzard and eliminated areas of higher pH where *Campylobacter* survives. This pH homogenization in the gizzard could prevent *Campylobacter* colonization of the cecum[17].

The second defense mechanism involves *Lactobacillus* spp., which predominantly inhabit chicken crops. *Lactobacillus* spp. are known to produce organic acids, such as lactic acid, acetic acid, hydrogen peroxide, and an antibacterial peptide, that inhibits *Campylobacter* growth under *in vitro* conditions[18–21]. However, *Campylobacter* inhibition has not been confirmed *in vivo* because animal experiments cannot maintain a sufficient residence time to kill *Campylobacter* in chicken crops. In our previous report[16], paddy rice fed to broiler chicks increased the residence time in the crop, resulting in an average retention time of 115 min, which allowed observation of the bactericidal activity of the inoculum in the crop. In this study, the survival

Received: February 18, 2024, Accepted: April 16, 2024
Available online: May 31, 2024
Correspondence: Mari Nishii, Kyoto Prefecture Agriculture Experiment Station, Ayabe-Shi 623-0221, Japan. (E-mail: m-nishii34@pref.kyoto.lg.jp) † Present address: Office YASUTOMI, Ayabe-Shi 623-0031, Japan

of *Campylobacter* remaining in broilers was evaluated by taking advantage of the increased residence time in broiler crops fed paddy rice.

Materials and Methods

Ethics statement

This study was approved by the Animal Use and Care Committee of Kyoto Prefectural Agriculture, Forestry, Fisheries, and Livestock Technology Center (approval number: 6-116).

Microorganisms, culture conditions, and preparation of the inoculum

Campylobacter jejuni GTC 03263, a strain used previously, was used in this study[17]. For the preparation of inoculum, a bacterial culture was revived from the stock bacterial suspension stored at −80 °C in the Micro Bank (Iwaki Co., Tokyo, Japan). Two micro beads were inoculated in 5 mL brain heart infusion (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and incubated at 37 °C for 48 h under microaerobic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen) and then 100 µL of the suspension was spread onto nutrient agar plates (Nissui). Plates were then incubated at 37 °C for 48 h under microaerobic conditions, and two platinum loops of the cultured bacteria were harvested after incubation and resuspended in 5 mL sterile saline solution. This suspension contained approximately 1×10^8 colony-forming units [CFU]/mL) and was used for *Campylobacter* oral inoculation.

A chicken crop is a diverticulum of the esophagus that temporarily stores the unsorted flow of ingested food[22]. This property enables the use of titanium dioxide (TiO_2), which is used as an indicator substance in digestion tests, to simultaneously monitor the dynamics of retention and excretion of dosed substances in the crop. A TiO_2-water suspension (0.5 g/mL) was prepared by mixing TiO_2 (Wako Pure Chemical Industries, Osaka, Japan) in sterile saline.

The oral inoculation used in this study was prepared by mixing the *Campylobacter* and TiO_2-water suspensions at a ratio of 1:9, and was stored in a plastic tube for several minutes before inoculation.

Experimental animals and the challenge model

Twenty 1-day-old female broiler chicks (Chunky) were raised in a flock-rearing area, measuring approximately 2 m^2 and covered with sawdust, in a windowless broiler house. The rearing area temperature was set at 34 °C on the first day and was gradually lowered to 25 °C until the chicks reached 14 days of age. The room temperature was maintained between 20–25 °C after 15 days of age. The chicks were fed a starter feed (crushed corn at a weight ratio of 65%) until 14 days of age (Table 1). The chickens were fed a diet containing 60% whole-grain paddy rice after 15 days of age (Table 1). The feedstuff did not contain any antimicrobials or coccidiostats. All chicks had free access to food and water throughout the study. The duration of light exposure was continuously controlled until 7 days of age, and then 20 h of light exposure per day was provided. All chicks were housed in an experimental cage at 26 d of age. The birds were confirmed to be culture-negative for *Campylobacter* prior to inoculation.

The prepared inoculum suspension (1 mL) was inoculated into the pharynx of all broilers using a 1 mL plastic syringe attached to a flexible tube. After inoculation, groups of four broiler chicks were randomly selected at 20-min intervals until 80 min and euthanized by anesthesia overdose (intravenous injection of sodium pentobarbital, Somnopentyl; Kyoritsu Pharmacy, Tokyo, Japan). The broiler crops were immediately excised with sterilized surgical scissors and the contents were transferred into 100 mL sterilized beakers. The weight of the crop contents was measured, and approximately 1 g of the contents was collected aseptically into sterile plastic tubes. The samples were stored in an icebox for approximately 2 h prior to bacterial detection. The remaining portion of the crop content was added to two volumes of deionized water in a 50 mL centrifuge tube and mixed using a vortex mixer for 1 min. The pH of the diluted mixture was repeatedly measured using a glass electrode pH meter (9615-10D; Horiba, Kyoto, Japan). The contents in the beaker were dried at 105 °C overnight using a ventilation dryer. The dried content was crushed using a food mill and subjected to quantitative determination of TiO_2 using the method described by Short *et al*.[23].

Enumeration of bacteria

For *Campylobacter jejuni* enumeration, the culture methods described below were used based on the fact that *Campylobacter* enters a viable but non-culturable state that has not been confirmed *in vivo*.

The preserved crop contents (1 g) were diluted 1:10 (w/v) using dilution anaerobic buffer solution (composition: KH_2PO_4, 4.5 g; Na_2HPO_4, 6.0 g; L-cysteine hydrochloride, 0.5 g; Tween 80, 0.5 g; and agar, 1 g in 1000 mL of purified water) in a plastic tube. The first suspension was serially diluted 10-fold with a diluted anaerobic buffer solution. The suspensions were spread onto modified charcoal cefoperazone deoxycholate agar (CCDA) plates (Oxoid, Basingstoke, United Kingdom), whereas modified *Lactobacillus* Selective agar (mLBS) was used for *Lactobacillus* sp. The CCDA medium consisted of a *Campylobacter* blood-free selective agar base (CM739; Oxoid) with a *Campylobacter* selective supplement (SR155; Oxoid) and a *Campylobacter* growth supplement (SR084; Oxoid). The plates were incubated at 42 °C under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2) for 48 h. The isolated colonies were confirmed to be *C. jejuni* using standard microbiological methods (International Standards Organization, 2006), including Gram staining, catalase and oxidase tests, specific spiral morphology, and corkscrew motility observed using phase-contrast microscopy. The number of bacteria in the samples of 60 min and 80 min after the inoculation was measured using an enrichment culture. Briefly, 1 g of crop content was added to 10 mL Preston broth (Nutrient broth No. 2 [CM67; Oxoid] supplemented with SR117, SR084, and 5% [vol/vol] lysed horse blood, Oxoid) and cultured at 42 °C under microaerobic conditions for 24 h. One platinum loop of Preston broth was plated onto a CCDA plate. The plates were incubated at 42 °C under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2) for 48 h. Colonies suspected to be *C. jejuni* were isolated and

Table 1. **Ingredient of the experimental diets**

Ingredients (%)	1 to 14 days	15 to 26 days
Ground corn	65	10
Paddy rice		60
Soybean meal	20	20
Fish meal (65% crude protein)	7	5
Corn gluten meal	2	
Soybean oil	3	2
Calcium carbonate	0.5	0.5
Tricalcium phosphate	0.6	0.6
Dicalcium phosphate	0.5	0.5
Manganese sulfate	0.015	0.015
Sodium chloride	0.25	0.19
DL-methionine	0.3	0.25
L-lysine HCl	0.5	0.4
Riboflavin	0.0004	0.00025
Copper sulfate	0.0005	0.001
Zinc sulfate	0.0005	0.004
Folacin	0.00004	0.00003
L-threonine	0.25	0.25
Choline chloride	0.05	
Calcium pantothenate	0.0004	
Nicotinamide	0.003	
Vitamin/mineral premix[a]	0.3	0.3
Calculated		
Crude protein (%)	21.1	17.5
Metabolizable energy (kcal/kg)	3,056	2,748

a) Vitamin and mineral premix including the following(per kg of the diet): retinol (retinyl acetate), 3,500,000 IU; cholecalciferol, 700,000 IU; vitamin E (DL-α-tocopheryl acetate), 600 mg; menadione, 250 mg; thiamine, 500 mg; riboflavin, 450 mg; pyridoxine, 350 mg; cyanocobalamin, 0.8 mg; nicotinamide, 1,700 mg; D-pantothenic acid, 750 mg; choline chloride, 35,000 mg; ZnCO3, 5,700 mg; MnSO4, 8,250 mg; FeSO4, 3,890 mg; CuSO4, 1,160 mg; CoSO4, 17 mg.

incubated on blood agar base No. 2 (Oxoid) containing 5% lysed horse blood. The isolated colonies were confirmed to be *C. jejuni* using standard microbiological methods (International Standards Organization, 2006), including Gram staining, catalase and oxidase tests, specific spiral morphology, and corkscrew motility observed using phase-contrast microscopy. *Lactobacillus* spp. were quantified using mLBS, i.e., prepared by supplementing LBS agar (BD, Becton Dickinson and Company, Sparks, MD, USA) with 0.8% Lab-Lemco Powder (Oxoid), 0.1% sodium acetate trihydrate, and 0.37% acetic acid. The plates were incubated at 38 °C for 48 h under anaerobic conditions, as described above, and the colonies were counted.

Modeling the Campylobacter survival curve

A crop nonselectively passes food components to the next segment[22]; therefore, the mixed suspension of *C. jejuni* and TiO_2 remains in the same ratio in the crop at any elapsed time. Therefore, the fraction of the amount of remaining TiO_2 in the crop to the dosed TiO_2 (TiO_2 in the crop/dosed TiO_2) may be substituted for the fraction of the remaining *C. jejuni* in the crop to the dosed *C. jejuni* (*C. jejuni* in the crop/dosed *C. jejuni*) in each sample of crop contents at the same elapsed time.

That is:

$$TiO_2 \text{ in the crop/dosed } TiO_2 = C.\ jejuni \text{ in the crop/dosed } C.\ jejuni \quad (1)$$

In the above formula, the units TiO_2 and *C. jejuni* present the weight and number of bacteria, respectively.

In addition, if the fraction of live *C. jejuni* to the remaining fraction (live or dead) in the crop at some time t after the challenge is defined as the survival fraction $S_{crop}(t)$, the number of live bacteria in every sample at some time t is expressed by the following formula:

$$\text{Live } C.\ jejuni \text{ in the crop} = C.\ jejuni \text{ in the crop} \times S_{crop}(t)$$

Substituting the above formula into formula (1) and arranging it:

$$S_{crop}(t) = (\text{live } C.\ jejuni \text{ in the crop/dosed } C.\ jejuni)/\ (TiO_2 \text{ in the crop/dosed } TiO_2) \quad (2)$$

The survival curve of $S_{crop}(t)$ is obtained by fitting the cumulative form of the Weibull distribution[24].

$$S_{crop}(t) = \exp(-bt^n) \quad (3)$$

where b and n are constants. b and n were derived using nonlinear least-squares analysis in Microsoft EXCEL (2010). The values of b and n are used to generate the frequency distribution using the following equations:

$$\frac{dS_{crop}(t)}{dt} = bnt^{n-1}\exp\left(-bt^n\right) \quad (4)$$

where $\frac{dS_{crop}(t)}{dt}$ is the frequency distribution of lethal events corresponding to t[25]. Other statistical parameters that characterize the distribution (mean, $\overline{t}$; variance, σ_t^2) are calculated using the following equations[24]:

$$\overline{t} = \left\{\Gamma\left[(n+1)/n\right]\right\}/b^{1/n} \quad (5)$$

$$\sigma_t^2 = \left\{\Gamma\left[(n+2/n)\right] - \left(\Gamma\left[(n+1)/n\right]\right)^2\right\}/b^{2/n} \quad (6)$$

where Γ is the gamma function. The mean t corresponds to the inactivation time on average with its variance, σ_t^2.

Statistical analyses

The Wilcoxon rank-sum test was used to test for significant differences in the survival fraction, $S_{crop}(t)$, of *C. jejuni* at the time of inoculation and at each time point.

Table 2. **Bacterial counts and pH in the crop contents dosed the mixed suspension of *C. jejuni* and Titanium dioxide at the time elapsed after inoculation (n=4)**

Time after inoculation (min)	*C. jejuni* (log10 cfu/g)	*Lactobacillus* spp. (log10 cfu/g)	pH
0	5.93	-	-
	5.87	-	-
	6.00	-	-
	5.85	-	-
20	5.88	7.69	5.49
	5.77	7.45	5.80
	5.93	6.48	4.81
	5.92	-	-
40	4.03	7.45	4.75
	5.12	-	-
	4.79	-	-
	4.66	-	-
60	3.74	8.98	4.73
	2.89	8.36	4.87
	ND	7.97	4.70
	ND	8.49	4.64
80	ND	8.67	4.80
	ND	8.72	4.70
	ND	8.54	4.66
	ND	8.92	4.58

ND: no detected colonies; -: not examined.

Results

Bacterial counts (*C. jejuni* and *Lactobacillus.* spp.), along with the pH of the crop contents sampled at 20-min intervals after inoculation, are presented in Table 2. *C. jejuni* was not detected in two out of the four samples at 60 min in the crop contents, but was not detected in any of the four samples at 80 min after inoculation, even using the enrichment culture method. When no *C. jejuni* was detected in a sample, the colony count of *C. jejuni* was deemed to be zero.

The TiO_2 residual ratio (TiO_2 in the crop/TiO_2 dose) and $S_{crop}(t)$ in the content of the crop sampled at each elapsed time are listed in Table 3. The TiO_2 residual ratio gradually decreased over time after inoculation, with 57% of the inoculated TiO_2 remaining in the crop after 60 min. In contrast, the survival fraction of the dosed *C. jejuni* in the crop ($S_{crop}(t)$) decreased 20 min–40 min after inoculation. A statistically significant difference was observed between $S_{crop}(0)$ and the survival fraction $S_{crop}(t)$ at 40, 60, and 80 min after inoculation using the nonparametric test (Table 3).

Equation (1) was used to calculate the value of $S_{crop}(t)$ for each sample, and the parameters b and n were determined to be $0.02613^{17.6826}$ and 17.6826, respectively, using the least-squares method. The survival fraction $S_{crop}(t)$ for each elapsed time and the survival curve obtained by substituting parameters b and n into Equation (3) are depicted in Fig. 1. The frequency distribution of the survival fraction $S_{crop}(t)$ is obtained by substituting these parameters into Equation (4), as illustrated in Fig. 2.

Furthermore, the mean survival time ($\overline{t}$) and variance (σ_t^2) of the distribution of the survival fraction $S_{crop}(t)$ were calculated to be 37.1 min from equation (5) and 6.74 min^2 from equation (6), respectively. The decrease in the survival fraction from 30 min to 40 min after inoculation is shown in Fig. 1. The lethal *C. jejuni* events between 30 min and 40 min after crop inoculation are shown in Fig. 2.

The number of *Lactobacillus* spp. in the crop contents of chickens inoculated with the mixed suspension varied in the range of 7.45–8.71 log CFU/mL at each elapsed time. The bacterial load is similar to that of previous reports[26–28]. The pH of the crops measured at each elapsed time point ranged from 4.69–5.37.

Discussion

The levels of remaining TiO_2 in the crop at 20, 40, 60, and 80 min after inoculation were 89%, 71%, 57%, and 66% of the inoculated amount, respectively (Table 3). Most of the TiO_2 persisted in the crop for 80 min, indicating that the inoculated *C. jejuni* also remained in a live or dead state at approximately the same rate in the crop. However, the survival fraction of *C. jejuni* remaining in the crops was 11% at 40 min, only 1% at 60 min, and was undetectable at 80 min using the culture method

Table 3. **The residual ratio of Titanium dioxide (TiO_2) in the crop contents and survival fraction (S_{crop}(t)) of the elapsed time after inoculation of the mixed susupension of *C. jejuni* and Titanium dioxide**

	Time after inoculation (min)				
	0	20	40	60	80
TiO_2 residual ratio	1.00 ± 0.00	0.89 ± 0.09	0.71 ± 0.12	0.57 ± 0.17	0.66 ± 0.05
$S_{crop}(t)^c$	1.00 ± 0.14^a	1.13 ± 0.11^a	0.11 ± 0.07^b	0.01 ± 0.01^b	0.00 ± 0.00^b

Data are presented as mean ± SD (n=4)

a-b) Means within the same row with different superscripts are significantly different ($p<0.01$)

c) $S_{crop}(t)$ = (lived *C. jejuni* in the crop/dosed *C. jejuni*)/ (TiO_2 in the crop/dosed TiO_2)

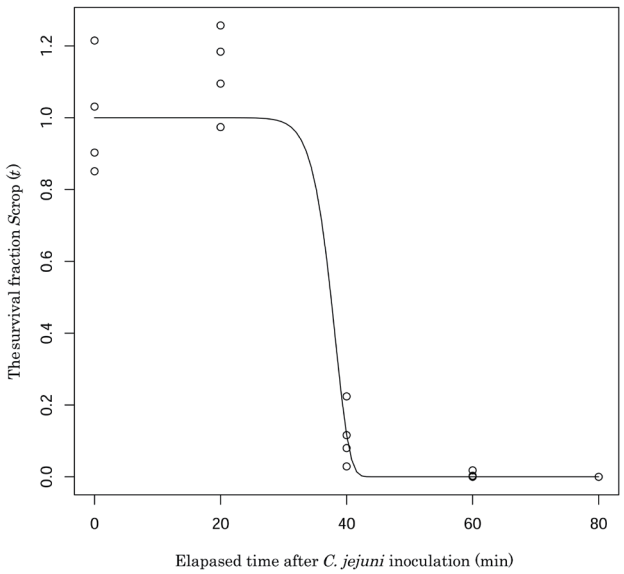

Fig. 1. **The survival fraction $S_{crop}(t)$ and the survival curve of $S_{crop}(t)$ in the crop for each elapsed time after *C. jejuni* inoculation (white dots, n = 4).**

The survival curve of $S_{crop}(t)$ is calculated from equation (2); $S_{crop}(t) = \exp(-bt^n)$, n = 17.6826, b = 0.02613^n.

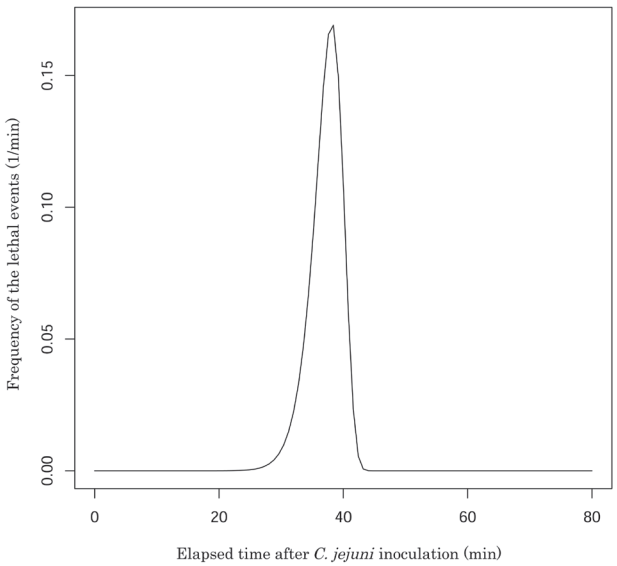

Fig. 2. **The frequency distribution of lethal events corresponding to time after *C. jejuni* inoculation.**

The frequency distribution ($\frac{dS_{crop}(t)}{dt}$) is calculated from equation (3); $\frac{dS_{crop}(t)}{dt} = bnt^{n-1}\exp\left(-bt^n\right)$, n = 17.6826, b = 0.02613^n.

(Table 3). Most of the inoculated *C. jejuni* died in the crop before entering the next segment. These data indicated that a lethal event occurred intensively over a certain period, between 30 min and 40 min, as revealed in Figs. 1 and 2. The mean survival time of *C. jejuni* in the crop was calculated to be 37.1 min. These data suggest that *C. jejuni* remaining for more than 40 min in a chicken crop died.

No lethal events occurred up to 20 min after inoculation of the crop (Figs. 1, 2). Therefore, these results indicate that a crop residence time of less than 20 min cannot prevent *C. jejuni* infection. Broilers raised with free access to nutritious feeds rarely store ingested feeds in their crops[29,30]. For example, 78% of *ad libitum*-fed birds store less than 5 g dry matter of feed in the crop[29]. Additionally, a small amount of storage in the crop corresponds to a short retention time. The feed retention time in the crop was only 5–7 min in broilers with free access to feed, as reported in previous studies[31,32]. Therefore, if broilers are provided with conventional free feeding, the short residence time of crop contents will promote early passage to the next segment and allow *C. jejuni* to survive. Thus, the residence time of the crop content must be treated as an important factor affecting the bactericidal role of the crop.

Lactobacillus inhibits the growth of co-cultivated *Campylobacter in vitro*[18–21]. In this experiment, *Lactobacillus* formed the dominant bacterial flora (10^6–10^8 CFU/g) at any time after oral inoculation with *Campylobacter*, demonstrating the qualitative bactericidal activity of *Lactobacillus* in broiler crops. The crop showed bactericidal activity (89% of the inoculated *Campylobacter* was eliminated in 40 min and 99% in 60 min), but no such bactericidal activity was observed in *in vitro* experiments in which *Campylobacter* and *Lactobacillus* were co-cultivated[33] or in an exposed substrate of organic acids and bacteriocins produced by *Lactobacillus*[18–21]. However, the planktonic phase formed using broth media in these reports seems to be quite different from the co-cultivated phase chicken crops in the present experiment, because it has been observed that *Lactobacillus* attached to epithelial cells on the inner wall of the crop produces exopolysaccharide and forms biofilms[34]. Stationary biofilms of lactic acid bacteria likely inhibit pathogenic bacterial invasion through spatial or nutritional competitive exclusion[35]. Therefore, this experiments using chicken crops suggest that antimicrobial metabolites (organic acids or antibacterial peptides) produced by *Lactobacillus* and spatial or nutritional competitive exclusion by *Lactobacillus*-formed biofilms might enhance the bactericidal effect against challenged *C. jejuni.*

These data revealed that the frequency of *C. jejuni* death occurred intensively between 30 min and 40 min after broiler crop inoculation. Further investigations simulating the spatial and microbial characteristics of chicken crops are required to elucidate the factors contributing to the intense elimination of *C. jejuni* from the crop.

Acknowledgments

We thank former Professor Dr. Takayuki Ezaki, Graduate

School of Medicine, Gifu University, for providing the *Campylobacter* strain GTC 03263 (GTC: Gifu Type Culture Collection) used in this study.

Author Contributions

Mari Nishii conducted the experiments and drafted the original manuscript. Masaharu Yasutomi analyzed the data. Both authors critically discussed and reviewed the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] World Health Organization. Risk assessment of *Campylobacter* spp. in broiler chickens: technical report. WHO press. Geneva, Switzerland, 2009.

[2] Wagenaar JA, Newell DG, Kalupahana RS and Mughini-Gras L. *Campylobacter*: animal reservoirs, human infections, and options for control. Zoonoses–infections affecting humans and animals: focus on public health aspects. Cham: Springer, Dordrecht, Netherlands, 159–177, 2015.

[3] Igwaran A and Okoh AI. Human campylobacteriosis: A public health concern of global importance. Heliyon, **5**: e02814. 2019. https://doi.org/10.1016/j.heliyon.2019.e02814, PMID:31763476

[4] Hald B, Skovgård H, Bang DD, Pedersen K, Dybdahl J, Jespersen JB and Madsen M. Flies and *Campylobacter* infection of broiler flocks. Emerg Infect Dis, **10**: 1490–1492. 2004. https://doi.org/10.3201/eid1008.040129, PMID:15496257

[5] French N, Barrigas M, Brown P, Ribiero P, Williams N, Leatherbarrow H, Birtles R, Bolton E, Fearnhead P and Fox A. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ Microbiol, **7**: 1116–1126. 2005. https://doi.org/10.1111/j.1462-2920.2005.00782.x, PMID:16011749

[6] Meerburg BG, Jacobs-Reitsma WF, Wagenaar JA and Kijlstra A. Presence of *Salmonella* and *Campylobacter* spp. in wild small mammals on organic farms. Appl Environ Microbiol, **72**: 960–962. 2006. https://doi.org/10.1128/AEM.72.1.960-962.2006, PMID:16391145

[7] Backhans A and Fellström C. Rodents on pig and chicken farms – a potential threat to human and animal health. Infect Ecol Epidemiol, **2**: 17093. 2012. https://doi.org/10.3402/iee.v2i0.17093, PMID:22957130

[8] Yogasundram K, Shane SM and Harrington KS. Prevalence of *Campylobacter jejuni* in selected domestic and wild birds in Louisiana. Avian Dis, **33**: 664–667. 1989. https://doi.org/10.2307/1591142, PMID:2619661

[9] Vlahović K, Matica B, Bata I, Pavlak M, Pavičić Ž, Popović M, Nejedli S and Dovč A. Campylobacter, salmonella and chlamydia in free-living birds of Croatia. Eur J Wildl Res, **50**: 127–132. 2004. https://doi.org/10.1007/s10344-004-0052-1

[10] Mohan V. The role of probiotics in the inhibition of *Campylobacter jejuni* colonization and virulence attenuation. Eur J Clin Microbiol Infect Dis, **34**: 1503–1513. 2015. https://doi.org/10.1007/s10096-015-2392-z, PMID:25934376

[11] Van Deun K, Haesebrouck F, Heyndrickx M, Favoreel H, Dewulf J, Ceelen L, Dumez L, Messens W, Leleu S, Van Immerseel F, Ducatelle R and Pasmans F. Virulence properties of *Campylobacter jejuni* isolates of poultry and human origin. J Med Microbiol, **56**: 1284–1289. 2007. https://doi.org/10.1099/jmm.0.47342-0, PMID:17893162

[12] Coward C, van Diemen PM, Conlan AJK, Gog JR, Stevens MP, Jones MA and Maskell DJ. Competing isogenic *Campylobacter* strains exhibit variable population structures in vivo. Appl Environ Microbiol, **74**: 3857–3867. 2008. https://doi.org/10.1128/AEM.02835-07, PMID:18424530

[13] Sahin O, Kassem II, Shen Z, Lin J, Rajashekara G and Zhang Q. *Campylobacter* in poultry: ecology and potential interventions. Avian Dis, **59**: 185–200. 2015. https://doi.org/10.1637/11072-032315-Review, PMID:26473668

[14] Moen B, Rudi K, Svihus B and Skånseng B. Reduced spread of *Campylobacter jejuni* in broiler chickens by stimulating the bird's natural barriers. J Appl Microbiol, **113**: 1176–1183. 2012. https://doi.org/10.1111/j.1365-2672.2012.05404.x, PMID:22817452

[15] Engberg RM, Hedemann MS, Steenfeldt S and Jensen BB. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult Sci, 83: 925-938. 2004. https://doi.org/10.1093/ps/83.6.925

[16] Nishii M, Yasutomi M and Sone Y. Effects of a whole-grain paddy rice diet on the pH distribution in the gizzard and retention time of digesta in the crop of broiler chicks. J Poult Sci, 53: 181-191. 2016. https://doi.org/10.2141/jpsa.0150142

[17] Nishii M, Yasutomi M and Sone Y. Inhibitory Effect of whole grain paddy rice feeding on the colonization of *campylobacter jejuni* in the cecum of broiler chicks. J Poult Sci, 52: 312-317. 2015. https://doi.org/10.2141/jpsa.0140193

[18] Chaveerach P, Lipman LJA and van Knapen F. Antagonistic activities of several bacteria on in vitro growth of 10 strains of *Campylobacter jejuni/coli.* Int J Food Microbiol, **90**: 43–50. 2004. https://doi.org/10.1016/S0168-1605(03)00170-3, PMID:14672829

[19] Stern NJ, Svetoch EA, Eruslanov BV, Perelygin VV, Mitsevich EV, Mitsevich IP, Pokhilenko VD, Levchuk VP, Svetoch OE and Seal BS. Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. Antimicrob Agents Chemother, **50**: 3111–3116. 2006. https://doi.org/10.1128/AAC.00259-06, PMID:16940109

[20] Alemka A, Clyne M, Shanahan F, Tompkins T, Corcionivoschi N and Bourke B. Probiotic colonization of the adherent mucus layer of HT29MTXE12 cells attenuates *Campylobacter jejuni* virulence properties. Infect Immun, **78**: 2812–2822. 2010. https://doi.org/10.1128/IAI.01249-09, PMID:20308300

[21] Wang G, Zhao Y, Tian F, Jin X, Chen H, Liu X, Zhang Q, Zhao J, Chen Y, Zhang H and Chen W. Screening of adhesive lactobacilli with antagonistic activity against *Campylobacter jejuni.* Food Control, **44**: 49–57. 2014. https://doi.org/10.1016/j.foodcont.2014.03.042

[22] Vergara P, Ferrando C, Jiménez M, Fernández E and Goñalons E. Factors determining gastkointestinal transit time of several markers in the domestic fowl. Q J Exp Physiol, **74**: 867–874. 1989. https://doi.org/10.1113/expphysiol.1989.sp003357, PMID:2512590

[23] Short FJ, Gorton P, Wiseman J and Boorman KN. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim Feed Sci Technol, **59**: 215–221. 1996. https://doi.org/10.1016/0377-8401(95)00916-7

[24] Peleg M and Cole MB. Reinterpretation of microbial survival curves. Crit Rev Food Sci Nutr, **38**: 353–380. 1998. https://doi.org/10.1080/10408699891274246, PMID:9704188

[25] Aragao GMF, Corradini MG, Normand MD and Peleg M. Evaluation of the Weibull and log normal distribution functions as survival models of *Escherichia coli* under isothermal and non isothermal conditions. Int J Food Microbiol, **119**: 243–257. 2007. https://doi.org/10.1016/j.ijfoodmicro.2007.08.004, PMID:17869362

[26] Guan LL, Hagen KE, Tannock GW, Korver DR, Fasenko GM, *et al.* Detection and identification of *Lactobacillus* species in crops of broilers of different ages by using PCR-denaturing gradient gel electrophoresis and amplified ribosomal DNA restriction analysis. Appl Environ Microbiol, **69**: 6750–6757. 2003.

[27] Józefiak D, Rutkowski A, Jensen BB and Engberg RM. The effect of β-glucanase supplementation of barley- and oat-based diets on growth performance and fermentation in broiler chicken gastrointestinal tract. Br Poult Sci, **47**: 57–64. 2006. https://doi.org/10.1080/00071660500475145, PMID:16546798

[28] Rehman HU, Vahjen W, Awad WA and Zentek J. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Arch Anim Nutr, **61**: 319–335. 2007. https://doi.org/10.1080/17450390701556817, PMID:18030916

[29] Svihus B, Sacranie A, Denstadli V and Choct M. Nutrient utilization and functionality of the anterior digestive tract caused by intermittent feeding and inclusion of whole wheat in diets for broiler chickens. Poult Sci, **89**: 2617–2625. 2010. https://doi.org/10.3382/ps.2010-00743, PMID:21076099

[30] Classen HL, Apajalahti J, Svihus B and Choct M. The role of the crop in poultry production. Worlds Poult Sci J, **72**: 459–472. 2016. https://doi.org/10.1017/S004393391600026X

[31] Shires A, Thompson JR, Turner BV, Kennedy PM and Goh YK. Rate of passage of corn-canola meal and corn-soybean meal diets through the gastrointestinal tract of broiler and White Leghorn chickens. Poult Sci, **66**: 289–298. 1987. https://doi.org/10.3382/ps.0660289, PMID:3588495

[32] Rougière N and Carré B. Comparison of gastrointestinal transit times between chickens from D^+ and D^- genetic lines selected for divergent digestion efficiency. Animal, **4**: 1861–1872. 2010. https://doi.org/10.1017/S1751731110001266, PMID:22445147

[33] Chang MH and Chen TC. Reduction of *Campylobacter jejuni* in a simulated chicken digestive tract by Lactobacilli cultures. J Food Prot, **63**: 1594–1597. 2000. https://doi.org/10.4315/0362-028X-63.11.1594, PMID:11079707

[34] Greppi A, Asare PT, Schwab C, Zemp N, Stephan R and Lacroix C. Isolation and comparative genomic analysis of reuterin-producing *Lactobacillus reuteri* from the chicken gastrointestinal tract. Front Microbiol, **11**: 1166. 2020. https://doi.org/10.3389/fmicb.2020.01166, PMID:32670217

[35] Mgomi FC, Yang Y, Cheng G and Yang Z. Lactic acid bacteria biofilms and their antimicrobial potential against pathogenic microorganisms. Biofilm, **5**: 100118. 2023. https://doi.org/10.1016/j.bioflm.2023.100118, PMID:37125395