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Antimicrobial Effects of *Lactobacillus acidophilus* and *Lactobacillus reuteri* against *Campylobacter jejuni* in Fresh and Roasted Chicken Breast Fillets

Yasser Shahbazi [a*] | **Mahya Mozaffarzogh** [b]

[a] *Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.*
[b] *Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*

***Corresponding author: Yasser Shahbazi**
Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran. Postal code: 6715685414.
E-mail address: yasser.shahbazi@yahoo.com

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ABSTRACT

Background: The present study aimed to determine the antimicrobial effects of *Lactobacillus acidophilus* and *Lactobacillus reuteri* against *Campylobacter jejuni* in fresh and roasted chicken breast fillets.
Methods: Fresh and roasted chicken breast fillets were soaked in probiotic suspensions (11 log CFU/ml) and immersed in *C. jejuni* suspension (5 and 3 log CFU/ml). Afterwards, the fillets were placed in clean stomacher bags and refrigerated for 10 days until further analysis.
Results: The count of 5 log CFU/g in the fresh fillets treated with *L. acidophilus*, *L. reuteri*, *L. reuteri*, and *L. acidophilus* reached 3.45, 3.89, and 4.25 log CFU/g after 10 days of refrigerated storage, respectively. In the roasted fillets, the corresponding counts were estimated at 2.99, 3.54, and 3.92 log CFU/g, respectively. In addition, the inoculated 3 log CFU/g of *C. jejuni* reached 1.09-1.11 log CFU/g after the refrigerated storage of the fresh and roasted chicken breast fillets.
Conclusion: According to the results, the addition of *L. acidophilus* and *L. reuteri* to the fresh and roasted chicken breast fillets had inhibitory effects against the growth of *C. jejuni.*

1. Introduction

The consumption of chicken meat is highly common, and chicken meat production constitutes approximately 30% of total meat production in the world [1,2]. Today, the growing consumption of poultry meat (especially chicken) has necessitated the assurance of the food safety and quality properties of these products. A major challenge in this regard is the contamination of raw chicken meat with foodborne spoilage microorganisms and pathogens during slaughtering, processing, and storage [3].

Reports have suggested that more than 144 million pounds of raw chicken meat-based foods are spoiled due to contamination with microbial and chemical agent s in the United States [4]. This issue is often attributed to the intrinsic water contents, nutrient compounds, and cross-contamination through equipment and washing water [5]. Extensive research has been focused on the common chemical and microbial properties of fresh chicken meat, including the total viable count, psychrotrophic bacterial count (PTC), total volatile base nitrogen, and peroxide value as primary fresh and health quality indices [6,7]. *Campylobacter jejuni* is an emerging pathogen of poultry meat, which is directly involved in the development of human diseases and is a common commensal of poultry [8].

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According to a survey in this regard, *C. jejuni* is the third most common food safety risk, accounting for 150 disease outbreaks and 2,197 individual cases of foodborne diseases in the United States in 2001 [9]. In general, *C. jejuni* outbreaks in fresh food products have been reported in the United States, Canada, Asia, and the European Union [9].

Recently, food manufacturers have become interested in the technologies used for the inhibition and control of spoilage microorganism growth, such as heat treatment and incorporation of chemical synthetic additives [10,11]. Several novel approaches have been proposed in order to prevent the complications caused by heat in the organoleptic and nutritional properties of foodstuffs, reduce the application of chemical additives, maximize preservation quality, and minimize the risk of contamination with bacterial pathogens in fresh chicken meat; some of these methods include high hydrostatic pressure [1], modified atmosphere packaging [12,13], cold plasma treatment [4], biodegradable and edible films/coatings [7,14], and plant essential oils/extracts [3,5,15].

Although extensive research has been dedicated to reducing the risk of disease outbreaks and cross-contamination of fresh perishable foodstuffs using the mentioned novel technologies, more outbreaks have been reported worldwide, revealing the urgent need to develop more effective strategies for the reduction or inhibition of the risk of *C. jejuni* contamination [9,16].

Probiotic bacteria have numerous health properties for human and animals, including the reduction of bacterial, viral, and antibiotic diarrhea, irritable bowel syndrome, inflammatory bowel disease, lactose intolerance symptoms, atopic allergies, and low-density lipoprotein-cholesterol, recovery of ulcerative colitis, and improvement of the immune function [17-19]. Moreover, probiotic microorganisms have been used as additives for fresh foodstuffs (e.g., cheeses, dairy desserts, ice-cream, and yogurts); for instance, *Lactobacillus* spp. and *Bifidobacterium* spp. are used most commonly, while *Streptococcus thermophilus* and *Saccharomyces boulardii* are also applicable in this regard [20].

Recent findings have confirmed the antimicrobial activity of probiotic microorganisms in cottage cheese [21], smoked salmon [22], and raw chicken meat [23]. In addition, previous studies have indicated the inhibitory effects of *Lactobacillus* spp. and *Bifidobacterium* spp. against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Shigella flexneri, Salmonella typhimurium*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* [24,27].

To the best of our knowledge, no studies have been published regarding the inhibitory effects of probiotic microorganisms (e.g., *Lactobacillus acidophilus* and *Lactobacillus reuteri*) on the growth of *C. jejuni* in fresh and marinated chicken breast fillets. The present study aimed to determine the antimicrobial properties of *L. acidophilus* and *L. reuteri* against *C. jejuni* in fresh and roasted chicken breast fillets for 10 days during refrigerated storage at the temperature of 4 ± 1 ºC.

2. Materials and Methods

2.1. Preparation of Probiotic Microorganisms

*L. acidophilus (*PTCC 1643) and *L. reuteri* (PTCC 1655) were purchased from the culture archive of the Iranian Research Organization for Science and Technology (IROST) in Tehran, Iran. Each probiotic strain was selected from a single colony on the De Man, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany), cultured at the temperature of 37 ± 1 °C for 24 hours, and sub-cultured (0.1 ml, 37 ± 1 °C, 24 hours) in 10 milliliters of the brain heart infusion (BHI) broth (Merck, Darmstadt, Germany). Following that, the cultures were harvested via centrifugation (Sigma, Shropshire, UK), and the BHI in the cultures was removed via centrifugation at 5000×g for 15 minutes. The cultures were re-suspended in 10 milliliters of 0.1% buffered peptone water and enumerated on the MRS agar with the target concentration of 11 log CFU/ml after spread plating [28].

2.2. Preparation of Campylobacter jejuni

C. jejuni (NCTC 11168) was obtained from the Department of Microbiology, School of Medicine at Kermanshah University of Medical Sciences, cultured in the BHI broth at the temperature of 37 ± 1 °C overnight, and diluted to 5 (high) and 3 log CFU/ml (low) using a tenfold serial dilution in 0.1% peptone water for further experimentation [29].

2.3. Preparation of the Chicken Breast Fillets

Chicken breast fillets (weight: 250-350 g, width: 75-80 cm^2) were obtained from a local butchery in Kermanshah, Iran and subdivided into two groups of fresh and roasted fillets. In order to prepare the roasted fillets, the samples were soaked in 100 milliliters of home-made marinade containing olive oil (2.5%), saffron (0.1%), dried thyme (0.1%), chopped onion (0.2%), salt (0.1%), and red pepper (0.1%). Afterwards, the samples were removed from the marinade and left to leak for 30 minutes, followed by separation in storage in sterile stomacher bags (Interscience, Saint-Nom-la-Bretèche, France) at the temperature of 4 ± 1 °C until further analysis.

2.4. Inoculation of the Chicken Breast Fillets

Initially, the chicken fillets were sterilized using ultraviolet radiation in a biosafety cabinet class II for 30 minutes at room temperature [30]. Afterwards, the fillets were subdivided into four groups, including control (without probiotic microorganisms), samples containing L. *acidophilus*, samples containing *L. reuteri*, and samples containing *L. acidophilus* and *L. reuteri*.

For the inoculation of the chicken breast fillets, the samples were immersed in probiotic suspension (11 log CFU/g), dried at refrigerated temperature (4 ± 1 ºC) for four hours, and immersed in *C. jejuni* suspension (5 and 3 log CFU/ml) by shaking using a shaker for approximately 10 minutes in order to completely distribute the pathogenic bacterium. Afterwards, the fillets were removed from the culture suspension and dried in a clean place in the refrigerator at chilled condition for 30 minutes to obtain the desired bacterial attachment [7]. At the next stage, the fillets were placed in the sterile stomacher bags, preserved at refrigerated temperature (4 ± 1°C), and used for further analysis for 10 days (0, 2, 4, 6, 8, 10).

2.5. Enumeration of Pathogenic Microorganisms

For the enumeration of the inoculated *C. jejuni* (3 and 5 log CFU/g), 25 grams of the chicken breast fillets were weighed, mixed with 225 milliliters of 0.1% buffered peptone water for three minutes at room temperature, serially diluted tenfold in 0.1% buffered peptone water, and cultured on the Columbia agar (Merck, Darmstadt, Germany) [7]. Afterwards, the plates were incubated at the temperature of 44 ± 1 °C for 48 hours in microaerophilic conditions in a microbial candle jar containing Anaerocult® C gas pack (Merck, Darmstadt, Germany), and the results were expressed as log CFU/g of the chicken fillets.

2.6. Statistical Analysis

All the experiments were conducted in triplicate, and data analysis was performed in SPSS version 16. One-way analysis of variance (ANOVA) was used to determine the differences between the samples, and *P*-value of less than 0.05 was considered significant.

3. Results and Discussion

The outbreak rate of *C. jejuni* in fresh poultry products has been reported to be 70-75% in Iranian local markets [31, 32]. Figures 1a-b and 2a-b show our findings regarding the effects of probiotic microorganisms (*L. acidophilus* and *L. reuteri*) against *C. jejuni* in the fresh and roasted chicken fillets. As is depicted, the growth of *C. jejuni* reduced in the control samples (no probiotic microorganisms) from 5 to 4.61-4.76 log CFU/g and from 3 to 2.31-2.66 log CFU/g after 10 days of refrigerated storage. A similar trend has been reported by Duffy et al. as well (2006) [33]. Furthermore, the results obtained by Lee et al. (2016) [34] indicated that at the outset of the study, 7 log CFU/g *C. jejuni* was inoculated into chicken breast fillets, which decreased to 0.5 log CFU/g after one week of chilled storage; this is consistent with the results of the present study. In another study, Ala and Shahbazi (2019) [7] reported that the initial count of 5 log CFU/g of *C. jejuni* reduced to 4.11-4.35 log CFU/g in fresh chicken breast fillets after 14 days of refrigerated storage.

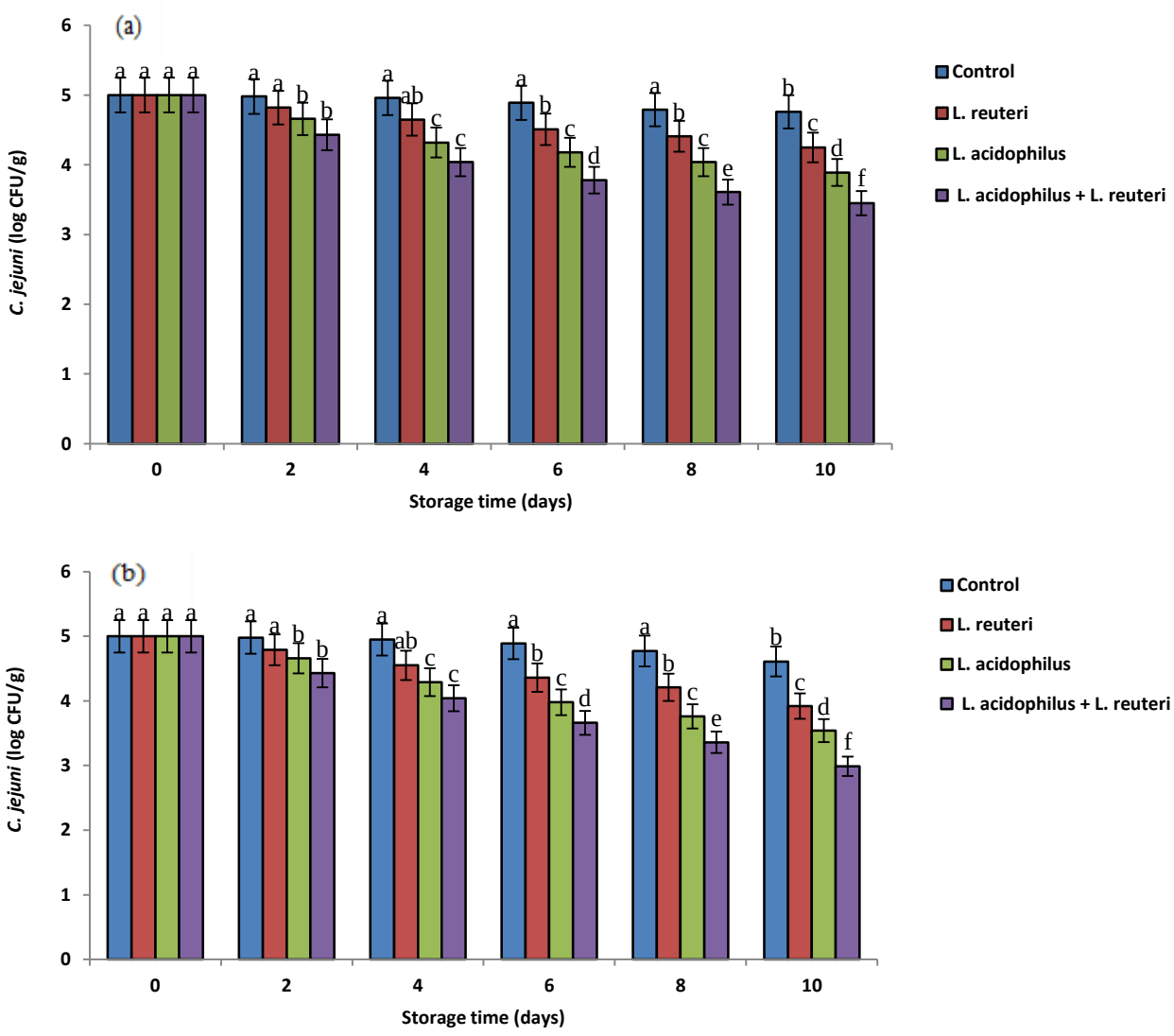

Figure 1: Antimicrobial Effects of *Lactobacillus acidophilus* and *Lactobacillus reuteri* against *Campylobacter jejuni* (5 log CFU/g) in a) fresh and b) roasted chicken breast fillets (Each number shows mean and standard deviation of three samples in various experiments; Different lower case letters indicate significant differences between sampling days; $P < 0.05$)

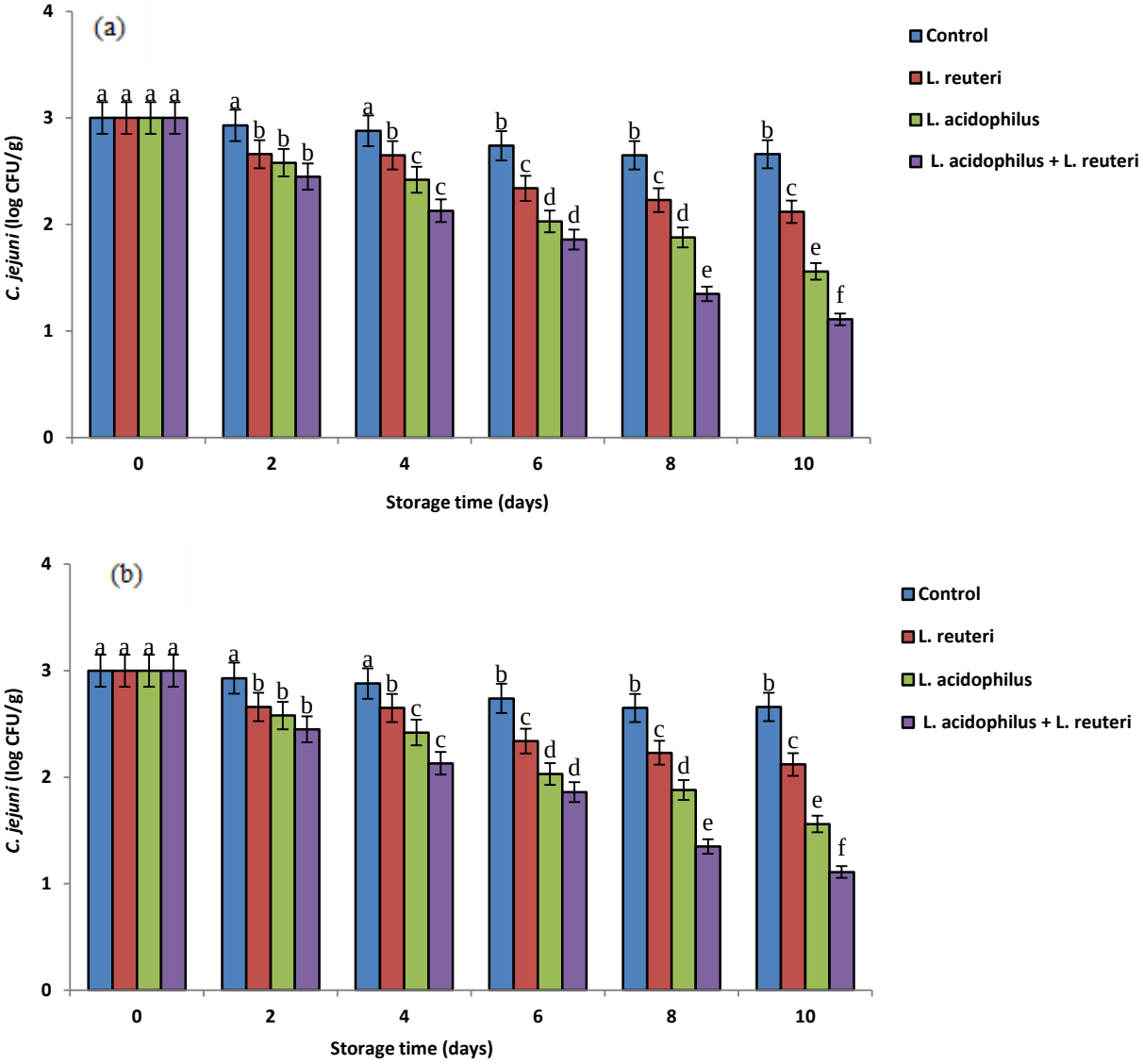

Figure 2: Antimicrobial Effects of *Lactobacillus acidophilus* and *Lactobacillus reuteri* against *Campylobacter jejuni* (3 log CFU/g) in a) fresh and b) roasted chicken breast fillets (Each number shows mean and standard deviation of three samples from various experiments; Different lower case letters indicate significant differences between sampling days; $P < 0.05$)

Moreover, our findings indicated that the used sauce for the roasted chicken fillets had no inhibitory effects against probiotic and pathogenic microorganisms. However, no significant difference was observed between the fresh and sauce-roasted chicken breast fillets in terms of the *C. jejuni* count ($P > 0.05$).

The findings of the current research confirmed that combined *L. acidophilus* and *L. reuteri* had the most significant inhibitory effects against *C. jejuni* in the fresh and roasted fillets, followed by *L. reuteri* and *L. acidophilus* (figures 1a-b & 2a-b). The count of 5 log CFU/g in the fresh fillets treated with L. acidophilus, *L. reuteri*, *L. reuteri*, and *L. acidophilus* reached 3.45, 3.89, and 4.25 log CFU/g after 10 days of chilled storage. As for the roasted fillets, the corresponding counts reached 2.99, 3.54, and 3.92 log CFU/g after the research, respectively. Interestingly, the inoculated 3 log CFU/g of *C. jejuni* reached 1.09-1.11 log CFU/g after the refrigerated storage period. In another research, Gialamas et al. (2010) [24] claimed that the use of Sodium caseinate film enriched with *Lactobacillus sakei* to the Tryptone soy agar laboratory medium and a food model system (fresh beef meat) inoculated with *L. monocytogenes* could significantly inhibit pathogen growth compared to the control samples. Similarly, Kaboosi (2011) [25] concluded that *L. rhamnosus* GG and *Bifidobacterium bifidum* could significantly inhibit the growth of pathogenic bacteria, including *S. aureus*, *E. coli*, *S. typhi*, and P. *aeruginosa*. In addition, Forestier et al. (2001) [26] reported that *L. casei* subsp. *rhamnosus* could decrease the growth of *S. flexneri*, *S. typhimurium*, *E. cloacae*, *P. aeruginosa*, and *E. faecalis.*

A critical property of probiotic microorganisms is their antagonistic ability against microbial pathogens through competition for exclusion, antibacterial aggregation or development of antibacterial constituents, including organic acids, bacteriocins (especially nisin), and hydrogen peroxide [35]. Numerous synergistic mechanisms have been confirmed for the presence of the natural

antimicrobial constituents that are produced by probiotic bacteria (e.g., bacteriocins), using the sequential suppression of a usual biochemical pathway and protective enzymes, combination of cell wall biological compounds, and cell wall active agents to increase the uptake of other antibacterial compounds [36, 37].

Several studies have indicated that probiotic microorganisms exert inhibitory effects against foodborne pathogenic bacteria and spoilage microorganisms. For instance, Ruiz Moyano et al. (2011) [38] reported that *L. reuteri* PL519 could significantly improve the shelf life of Salchichon, which is a traditional Iberian dry fermented sausage. On the other hand, the findings of Ghareeb et al. (2012) [39] demonstrated that *Enterococcus faecium*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, and *Lactobacillus reuteri* obtained from healthy poultry gut could suppress the growth of bacterial pathogens, especially C. jejuni. Moreover, Wang et al. (2014) [40] stated that bacterial strains such as *L. plantarum*, *L. acidophilus*, *L. casei, L. gasseri, L. reuteri,* and *L. salivarius* were effective in the antagonization of isolated *C. jejuni*.

4. Conclusion

According to the results, the incorporation of *L. acidophilus* and *L. reuteri* into the fresh and roasted chicken breast fillets had inhibitory effects against the growth of *C. jejuni*. Therefore, the designated treatments could be remarkably promising approaches to the increasing of the safety of raw and roasted chicken fillets. In conclusion, it is recommended that further investigation be conducted regarding the effects of *L. acidophilus* and *L. reuteri* on the shelf life improvement of fresh and processed foodstuffs.

Authors' Contributions

This article was carried out by all the authors. Y.Sh., and M.M., designed the manuscript and contributed to carry out data collection and data analysis and Y.Sh., and M.M., wrote the manuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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