

# Comparison between *E. coli* O157:H7 and *Bifidobacterium* spp. Activity in Almond Pudding Infant Supplemental Food

Rashin Sedighi<sup>1</sup>, Mehrdad Tajkarimi<sup>2</sup>, Salam A. Ibrahim<sup>2</sup>

<sup>1</sup>Maternal and Child Nutrition Program, University of California, Davis, USA; <sup>2</sup>Food Microbiology and Biotechnology Lab, North Carolina A & T State University, Greensboro, USA.

Email: rasedighi@ucdavis.edu

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## ABSTRACT

Almond pudding is a common traditional Iranian complementary food for infants after starting solid foods. *Escherichia coli* O157:H7 is one of the leading pathogenic microorganisms that cause serious foodborne disease in different populations including infants. The large intestine of breast-fed infants is colonized predominantly by bifidobacteria, which have a protective effect against acute diarrhea. The study objective of this research was to screen the survival characteristics of *E. coli* O157:H7 as well as four strains of *Bifidobacterium* subspecies (spp.) in almond pudding. The bacterial strains were studied after three and six hours of incubation at 37°C in-vitro. Luria-Bertani (LB) broth was used as a basic medium for both *Bifidobacterium* spp. and *E. coli* experiments in anaerobic and aerobic conditions, respectively. The viability of *Bifidobacterium* spp. increased from  $2.46 \pm 0.2$  to  $6.57 \pm 1.3 \log_{10}$  CFU/ml in low inoculum and from  $4.53 \pm 0.7$  to  $7.2 \pm 0.4$  in high inoculum experiments in 6 hours. However, the growth of *E. coli* O157:H7 from  $3.12 \pm 0.2$  to  $4.99 \pm 0.1 \log_{10}$  CFU/ml was significantly ( $P < 0.05$ ) lower compared to *Bifidobacterium* spp. The results illustrate impaired growth of *E. coli* O157:H7 and enhanced growth of *Bifidobacterium* spp. in almond pudding. The finding demonstrated that almond pudding in infant's diet may indirectly enhance the protection against survival and growth of *E. coli* O157:H7 by increasing the *Bifidobacterium* spp. populations in infant's gastrointestinal system.

**Keywords:** *Escherichia coli* O157:H7, *Bifidobacterium* spp., Infant Complementary Food

## 1. Introduction

Foodborne outbreaks, and investigating the causes of foodborne outbreaks or diseases, have not been a high priority in developing countries because of application of basic food safety rules in buying, cooking, storage and preservation combined with pathogen resistance of exposed populations [1,2]. Changes in food consumption habits might also affect the foodborne illness rate due to cultural effects. Ladies in shopping centers in developing countries are more concern about quality of the raw materials [3]. One of the common and high morbidity diseases in children younger than 5 years old is infectious gastroenteritis. New etiologies of diarrhea such as entero aggregative *E. coli* O157:H7 have been recognized in recent decades *E. coli* O157:H7 causes intestinal disorders ranging from mild diarrhea to hemorrhagic colitis and severe hemolytic uremic syndrome in infants [4-7]. The predominant enterohemorrhagic *E. coli* serotype af-

fecting humans in the United Kingdom and North America is *E. coli* O157:H7. In the US these infections have been associated primarily with contact with ruminant feces [8].

Several reports highlighted certain carbohydrates present in breast milk act as bifidus-specific growth factors supporting the implantation of these bacteria [9]. *Lactobacillus* and *Bifidobacterium* spp. are traditionally included in probiotic products to help protect against such effects [10]. *Bifidobacterium* spp. could act as a health promoting agent within the gastrointestinal tract and has been used as a probiotic for years [11].

Almond pudding is a traditional Iranian supplemental food for infants after 6 months of age. Generally, almond pudding supplement contains 10% completely smashed almond in a rice extract. Almonds (*Prunus dulcis* Miller D.A. Webb) are a rich source of phytochemicals and nutrients such as vitamin E. Almond consumption has been

associated with reduction in risk factors for chronic diseases and improvement of serum lipids [12]. Almonds contain polyphenols that may have protective characteristics against cancer and cardiovascular disease [13-17]. Polyphenols and phenolic compounds presented in different food materials have antimicrobial activity that reduce bacterial survival and growth in a natural environment [18-21]. Clinical studies show that nut consumption is associated with reduced incidence of cardiovascular disease, diabetes, and some forms of cancers [16, 17].

The objective of this study was to investigate characteristics of almond pudding on survival and growth of *E. coli* O157:H7 and *Bifidobacterium* spp.

## 2. Materials and Method

### 2.1. Bacterial Strains

Four strains of *E. coli* O157:H7 sub species, F4546 (alfalfa sprout isolate), H1730 (lettuce isolate), E0019 (beef isolate) and 944 (salami isolate) were used in this study. The strains were maintained on tryptic soy agar (TSA, Difco Laboratories, Becton Dickinson, Sparks, MD) slants at 4°C. The strains were transferred to fresh TSB before use. Then, cultures of each strain were grown separately in TSB and Luria-Bertani (LB) broth (Difco, Becton Dickinson, Sparks, MD) at 37°C overnight on a shaker and transferred onto the BHI plates at 24 h intervals.

Two strains of bifidobacterium (*Bifidobacterium* spp. 36, *Bifidobacterium* spp. 9) were used in this study. The strains were maintained on Lactobacilli MRS agar (Neogen, Lansing Michigan) slants at 4°C. The strains were transferred to fresh MRS broth before use. Then, cultures of each strain were grown separately in LB broth at 37°C and transferred onto the MRS plates at 24 h intervals under anaerobic conditions.

### 2.2. Inoculum Preparation

The mixture of *E. coli* strains was made to mimic the typical infection condition. We developed a model for simulating the exposure to *E. coli* O157:H7. The *E. coli* O157:H7 strains in a preliminary experiment with LB broth. The four strains were prepared by overnight culture of individual strains at 37°C, mixing the cultures together and centrifugation for 10 minutes at 8000 × g (Sorval RC 6 plus, Ashville, NC). The supernatant was decanted, and the resulting cell pellet was re-suspended in 0.1% peptone water (Becton Dickinson) to approximately 8 log<sub>10</sub> CFU/ml. Serial decimal dilutions were made to achieve an initial inoculum level of 4.0 log<sub>10</sub> CFU/ml before inoculation.

The *Bifidobacterium* strains were prepared by growing each strain at 37°C for 48 hours, mixing the cultures together followed by centrifugation for 10 minutes at 6000 × g (Sorval RC 6 plus, Ashville, NC). The supernatant was decanted, and the resulting cell pellet was re-suspended in 0.1% peptone water (Becton Dickinson) to approximately 8 log CFU/ml. Serial dilutions were made to achieve an initial inoculum levels of 3.0 log<sub>10</sub> CFU/ml (low inoculum) and 5.0 log CFU/ml (high inoculum) before inoculation.

### 2.3. Almond Pudding Preparation

Long grain rice (*Oryza sativa*) was boiled with equal volume of water for 30 minutes. The boiled water extract was filtered by a metallic kitchen filter and transferred to a sterile container.

Almond (*Prunus dulcis* Miller D.A. Webb) was boiled in water for 10 minutes. The whole almond (almond with skin treatment (AS)) and skinless almond (Almond treatment (AL)) was smashed thoroughly to make a homogeneous product. Almond pudding consisted of 10% (w/v) almond smash in 90% rice extract.

### 2.4. Laboratory Medium Experimental Treatments

LB broth was prepared and divided into three portions, to which almond pudding was added to levels of 0% (LB control), 50% LB with AS pudding (LB/AS) or 50% LB with AL pudding (LB/AL). Additionally, 100% AS and AL pudding was tested. The six hour period for treatments was selected to imitate the infant's gastrointestinal digestion period. These almond pudding preparations and the LB control were used in experiments for *E. coli* O157:H7.

*Bifidobacterium* spp. preparations at low and high inoculation were compared in similar preparations of almond pudding without skin. These treatments were Almond (AL) pudding alone, LB control, and LB/AL (50% LB). The culture condition for *Bifidobacterium* spp. has two inoculums and LB as a basic nutritional medium to mimic a closer condition to natural existence of *Bifidobacterium* spp. in the gastrointestinal system.

### 2.5. Bacterial Enumeration Experiments

Bacterial populations were determined for *E. coli* O157:H7 or *Bifidobacterium* spp. was according to the modified methods explained by Ibrahim *et al.* [22,23] by spreading samples of inoculated pudding or LB broth on BHI or MRS agar, respectively. Samples were withdrawn from inoculated almond pudding or LB broth at 0, 3 and 6 hours. Samples were serially diluted in 0.1% peptone water. Appropriate dilutions (100 µl) were spread onto dup-

licates of each type of agar plates and CFU counts were assessed after 24 hr.

### 2.6. Statistical Analysis

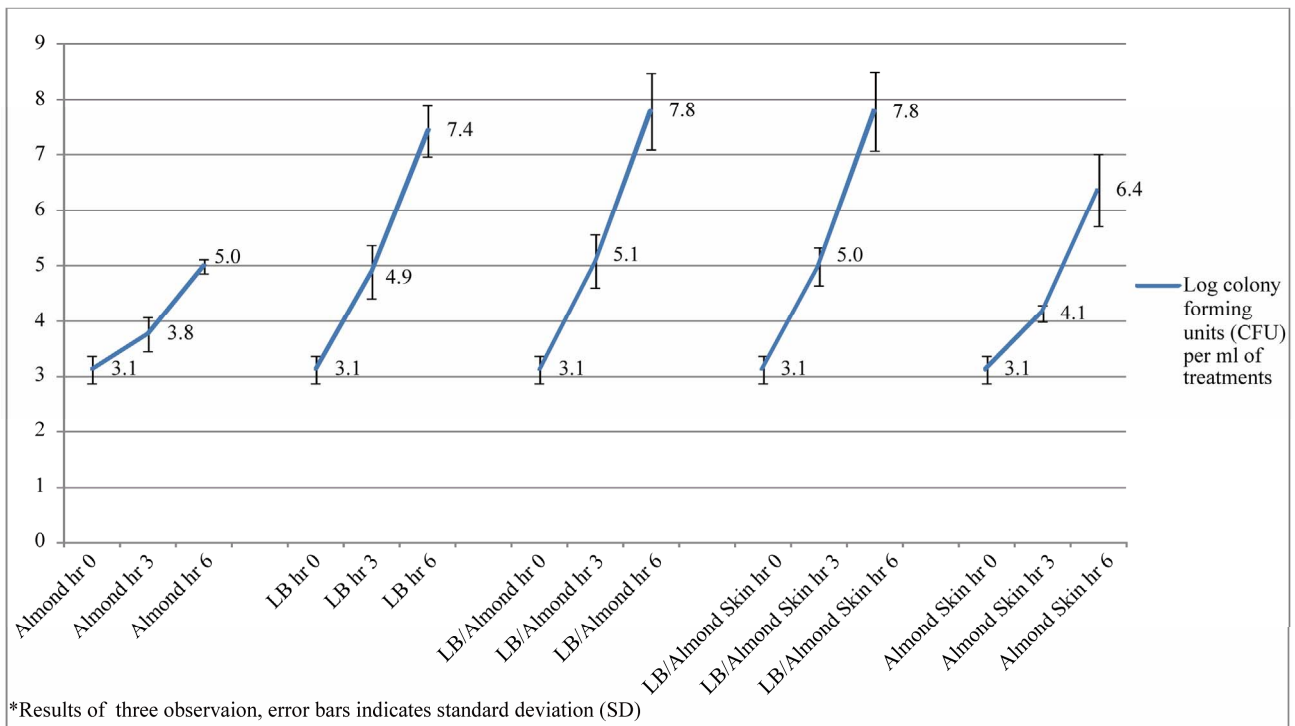
Each set of experimental tests was conducted four times to determine the effect of almond pudding on the survival and growth of the tested bacterial strains. One way ANOVA statistical analysis was performed for plate counts with a significance criterion of  $P \leq 0.05$  using PASW statistics 18 (SPSS, Chicago, Illinois). Statistical results were subjected to Bonferroni post hoc analysis for conservative analysis.

### 3. Results and Discussion

In this study, the growth of *E. coli* O157:H7 in almond pudding was investigated using almonds with skin and/or almonds without skin. *E. coli* in LB broth control culture increased about 2.3 fold after 6 h from  $3.12 \pm 0.2$  to  $7.45 \pm 0.5 \log_{10}$  CFU/ml of LB broth. This was similar to LB broth + AS or AL puddings where the number of bacteria increased 2.5 fold. The growth of *E. coli* O157:H7 in the presence of almond pudding with and without skin were not significantly different after 6 hours. However, there was significantly less growth of *E. coli* O157:H7 in almond pudding with or without almond skin compared to

LB treated samples ( $P < 0.05$ , **Figure 1**). According to **Figure 1**, there was no significant difference between *E. coli* O157:H7 treated samples in LB+ almond pudding ( $7.79 \pm 0.68 \log$  CFU/ml) and LB+ almond with skin pudding ( $7.78 \pm 0.71 \log$  CFU/ml) samples. There is also a significant ( $P < 0.05$ ) difference in *E. coli* growth between almond pudding with almond skin compared to almonds without skin that has been previously reported [13] and may need further study. However, our study focus was to investigate and create similar environment and growth condition to gastrointestinal (GS) system. It was assumed that the LB treated samples may be more similar to the GS system and mimic the nutritional condition within the GS system compare to the Almond itself. The result is also indicated significant difference between LB treated samples compare to non-LB treated samples.

The results from our study indicated that the limited growth of *E. coli* O157:H7 in almond pudding with and without skin might be due to interactions of other factors such as difference between nutrient supply and for the growth of *E. coli* O157:H7 in almond pudding compared to the LB treatments. Mandalari *et al.* 2010 indicate antimicrobial effect of purified almond skin extract. While another prominent ingredient in traditional Iranian pud-



**Figure 1. Survival and growth of *E. coli* O157:H7 experiments in log CFU with different treatments of Almond (AL), almond with skin (AS), Luria-Bertani broth (LB), Luria-Bertani broth with Almond (LB/AL) and Luria-Bertani broth with Almond Skin (LB/AS).**

ding is the rice extract, Kawakami *et al.* showed little *E. coli* growth, and no bactericidal effects of rice extracts after 2 hr incubation [24].

Others have shown that concentrates of polyphenols extracted from almond skin can inhibit *E. coli* O157:H7 growth at relatively high concentrations (Mandalri *et al.*, 2010). While the polyphenol content of the almond pudding was not measured it is likely that, given the method of preparation, the levels of polyphenols would not have been as concentrated. While another prominent ingredient in traditional Iranian pudding is the rice extract, Kawakami *et al.* showed little *E. coli* O157:H7 growth, and no bactericidal effects of rice extracts after a 2 hr incubation [24].

The conditions used to test *E. coli* O157:H7 and *Bifidobacterium* spp. growth were different based on the different growth condition between the two strains. Co-culture of the bacteria was not tested because of two reasons:

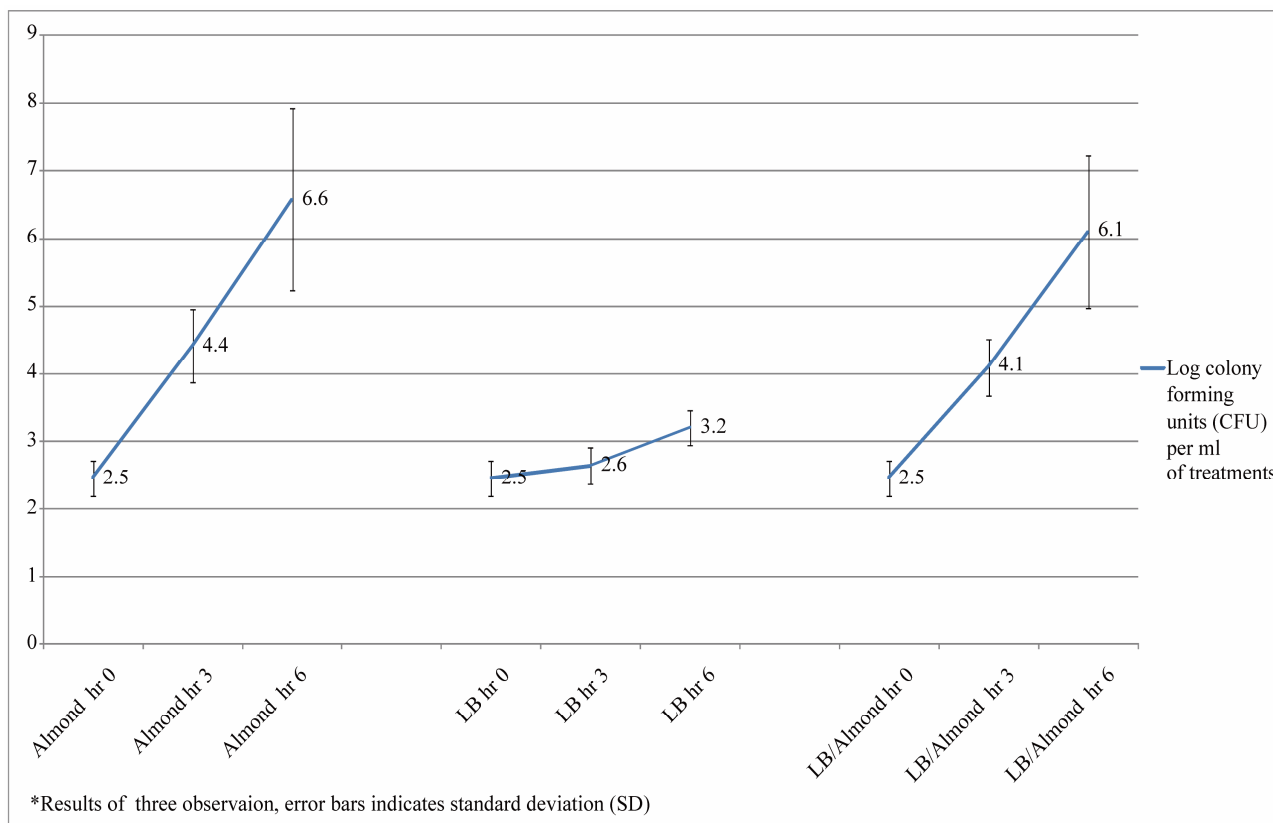
1) *Bifidobacterium* spp. growth required reducing agents such as ascorbic acid. Results of recent study by Tajkarimi *et al.* indicated the antimicrobial effect of ascorbic acid against *E. coli* O157:H7. This antimicrobial effect of ascorbic acid will also be enhanced by the lactic acid

which is also produced by *Bifidobacterium* spp. [25].

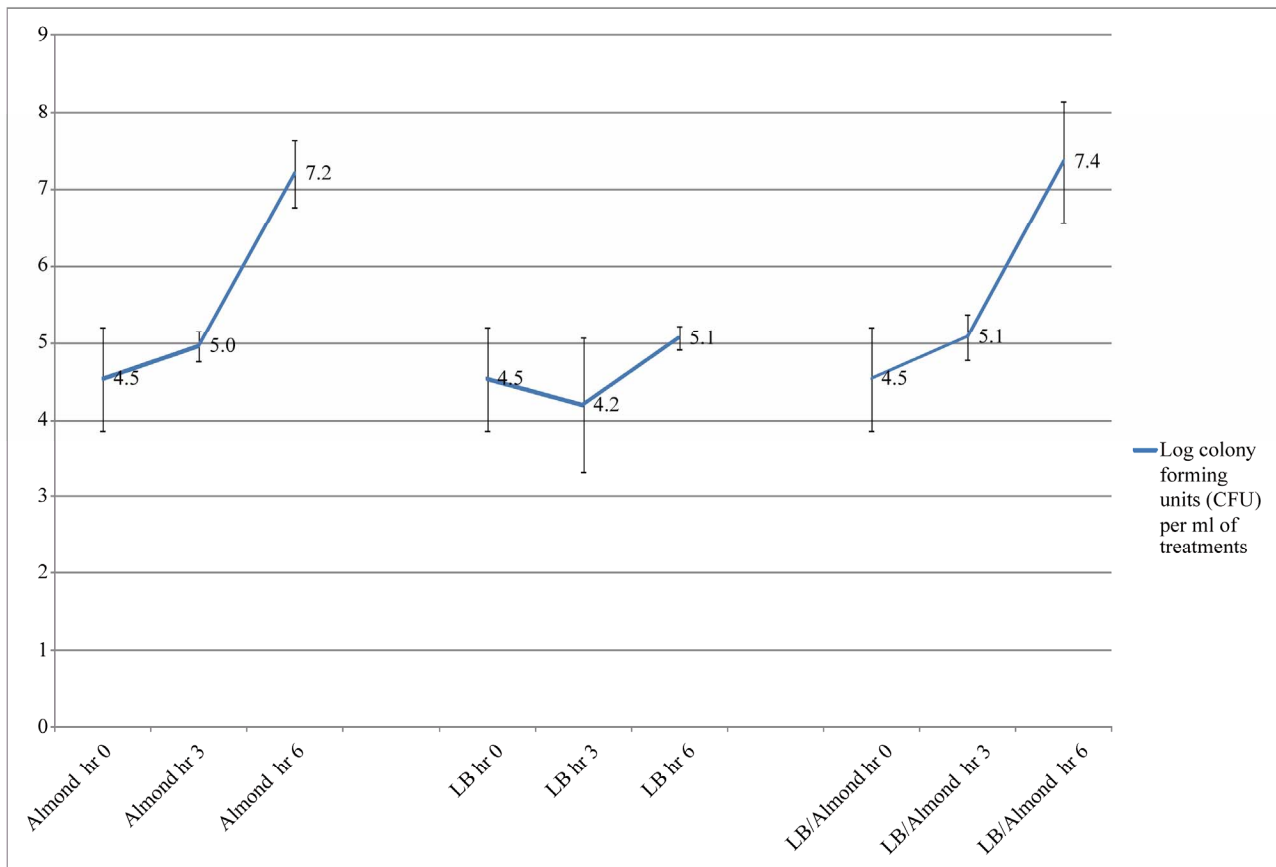
2) *Bifidobacterium* spp. functionality will be hindered under aerobic condition which is required for *E. coli* O157:H7 growth [26].

In another part of the study, the effect of almond pudding on the growth of *Bifidobacterium* spp. at high inoculation ( $4.54 \pm 0.7$  log CFU/ml) and low inoculation ( $2.54 \pm 0.3$  log CFU/ml) were compared. According to **Figure 2**, at low inoculation there was significant effect of the presence of almond pudding on *Bifidobacterium* spp. growth. After 6 hours of incubation almond pudding alone ( $6.08 \pm 1.12$  log CFU/ml) or almond pudding with LB ( $6.57 \pm 1.34$  log CFU/ml) had more *Bifidobacterium* growth compared to the LB control ( $3.20 \pm 0.24$  log CFU/ml) ( $P < 0.05$ ). A similar affect was seen when higher inoculation levels were used. **Figure 3** shows that *Bifidobacterium* spp. growth was greater in almond pudding ( $7.20 \pm 0.43$  log CFU/ml) or almond pudding with LB ( $7.35 \pm 0.79$  log CFU/ml) after 6 hours compared to the LB control ( $5.06 \pm 0.15$  log CFU/ml)) ( $P < 0.05$ ).

Overall comparison on *Bifidobacterium* spp. Experiments showed significant increase in growth in almond pudding without skin regardless of the presence of LB.



**Figure 2. Survival and growth of *Bifidobacterium* experiments in log FU (low inoculum) with different treatments of Almond (AL), Luria-Bertani broth (LB) and Luria-Bertani broth with Almond (LB/AL)\*.**



**Figure 3. Survival and growth of *Bifidobacterium* experiments in log CFU (high inoculum) with different treatments of Almond (AL), *Luria-Bertani* broth (LB) and *Luria-Bertani* broth with Almond (LB/AL)\*.**

Intestinal micro flora contains complex ecosystem with at least 50 bacteria genera and several hundreds of species. Comprising several hundred species, the majority of these bacteria reside in the colon and some are believed to bring health and therapeutic benefits to the host. *Bifidobacterium* spp. is the safest genera and act as an opportunistic bacterium [11]. There is strong evidence of the effect of intestinal flora including bifidobacteria on human health. Bifidobacteria appear to reduce the incidence of traveler’s diarrhea. They are also reportedly antagonistic towards pathogens in adults and especially in infants [27,28]. Carbohydrates can be used as growth elements for probiotics inside the intestinal tract and it could indirectly inhibit the growth of food borne pathogens including *Escherichia coli*, *Campylobacter jejuni* and *Salmonella enteritidis* [10]. Almond is a rich source of 22% protein and 21% carbohydrates including oligosaccharides [29,30] oligosaccharide are a desirable sources as a prebiotic to promote the growth of gastrointestinal beneficial microorganisms such as *Bifidobacterium* spp. and *Lactobacilli* [31,32] oligosaccharides are a third

component of human milk after lactose and lipids which are mainly responsible to dominate development of microbiota including bifidobacteria [33]. Further studies need to investigate the interaction and quantitative competition between both *E. coli* O157:H7 and *Bifidobacterium* spp. *in-vitro* and *in-vivo* experiments.

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