The Effect of a Fermented Probiotic, the Kefir, on Intestinal Flora of Poultry Domesticated Geese (Anser anser)

H. YAMAN*, Z. ULUKANLI1, M. ELMALI1, Y. UNAL3

SUMMARY

The objective of the present study was to determine the effects of kefir as a probiotic on the microbiology of gosling faeces in comparison with a control diet for 5 weeks and the 30 day old birds were divided into 3 groups (10 geese by group) according to the kefir supplementation in the drinking water: 0% for the control group, 0.20% in the group 1 and 0.50% in the group 2. Kefir significantly affected the faecal bacteria population by increasing Lactobacilli spp (p<0.01 in the group 2), and total aerobic bacteria populations (p<0.05 in the group 1 and p<0.01 in the group 2) and by decreasing the populations of Enterobacteriaceae (p<0.05 in the group 2), and coliforms (p<0.05 in the group 1 and 2). The Enterococci, Staphylococci and the yeast populations were slightly but not significantly, diminished in faeces of supplemented birds. The results of this study provided preliminary information that kefir Lactobacilli spp.-yeast may be a useful candidate as a competitive exclusion (CE) preparation to improve intestinal microflora of poultry and lead to better carcass hygiene.

Keywords : probiotic, fermented milk (kefir), geese, Lactobacilli, yeast

Introduction

Animal health and growth performance are affected by many factors such as diet, stress, antibiotics and modern husbandry practices. The gastro-intestinal microflora of healthy birds would be maintained stable by using antibiotics in preventive dosages. However, it is also argued that antibiotics may have indirect adverse side effects with implications for human health through consumption of food animal origin. Increased bacterial resistance to antibiotics in humans has caused an increased public and governmental interest for eliminating sub-therapeutic use of some antibiotics in livestock. To decrease the use of antibiotics, the use of probiotics may be considered as one of alternative approaches to replace chemical additives by lactic acid bacteria. These bacteria have been consumed in fermented foods and feeds for several centuries without any obvious adverse effects [16].

Carcass contamination and cross contamination between carcasses in a poultry processing plant generally result when intestinal contents are released from the birds [53]. The intestinal microflora population is a complex ecosystem composed of a large variety of bacteria and the intestinal tract of chickens are well established as reservoirs for pathogenic bacteria [2, 29, 31]. There is a great deal of interest in the possibility of altering the intestinal microflora in a beneficial way for the health of the host. Evidence suggests that many diseases caused by pathogenic bacteria invading the digestive tract could have been prevented if proper intestinal flora were maintained [17, 56]. Lactobacilli may play a role in the prevention of harmful bacteria invading and populating the digestive tract in the chicken [16, 26] and probiotic cultures can potentially improve the safety of poultry in the human supply by decreasing consumer exposure to enteric pathogens [37].

At present, many micro organisms and yeast are available as commercial probiotic products in the market [14, 18, 35, 36]. These products either contain a single strain or a mixture of cultures. Although effective cultures have been produced and available, air tolerant and inexpensive culture is needed to use in practice [4]. Most commercial veterinary probiotic preparations are not accurately represented by label claims and most products may have contained low concentrations of viable organisms [52]. Some poultry feed already contains probiotic bacteria, but as an undefined mixture, which gives inconsistent results.

* Corresponding : Dr. Mehmet ELMALI - Department of Food Hygiene and Technology, Faculty of Veterinary Science, University of Kafkas, Kars, Turkey.
1Department of Biology, Faculty of Science and Arts, University of Kafkas, Kars, Turkey.
2Department of Animal Nutrition, Veterinary Science, University of Kafkas, Kars, Turkey.
3Department of Food Hygiene and Technology, Faculty of Veterinary Science, University of Kafkas, Kars, Turkey.

Corresponding : Mehmet ELMALI
Tel: 00 90 474 242 68 00-06/1124 - Fax: 00 90 474 242 68 53 - Email: ELMALI25@hotmail.com
To date, most of research with probiotics has been limited to chicken and very little has been done with other species of poultry. Likewise, most of researches have focused on the effect of *L. acidophilus* milk and/or yoghourt. Research and information about the effects of kefir is not easily available. Furthermore, studies on the influence of microbial feed supplements, in particular lactic acid bacteria, have focused on their growth promoting effects on poultry and less attention has been given on their effects on intestinal microflora populations, which they are considered as indicators of faecal contamination [3, 8, 9, 13].

The aim of this study was to determine the effect of a probiotic, i.e. fermented milk prepared easily with kefir grains, on some intestinal microflora populations in geese. Kefir was chosen because it is easy to make and it is known to be safe for human use, which is major importance because geese will be used for human consumption.

**Materials and methods**

The present work was carried in the research farm of Kafkas University between May and June, 2004. Three groups of 2 weeks old female goslings (n = 10 in each group) were housed and kept individually in cages and did not have access to swimming water. They were fed with a starter diet with 22% crude proteins (CP) and 2100 kcal/kg metabolisable energy. Diet composition is given in Table 1. Kefir beverage was added to drinking water at a concentration of 0.0% (v/v) (Control group), 0.20% (v/v) (Group 1) and 0.50% (v/v) (Group 2) starting on day one during six weeks. Both food and water were offered *ad libitum*.

**Preparation of kefir beverage**

Kefir grains were added to sterilize UHT (Ultra High Temperature) whole milk at ratio of 1:50 (w/v) [28]. After incubation at 20-22 °C for 24 h., the grains were filtered through a sieve and the remaining kefir beverage was used as a dietary supplement in drinking water for goslings during the course of experimental work. To determine the total lactic acid flora, serial 10-fold dilutions of kefir beverage were spread out on MRS (Man Rogosa Sharpe, Merck, Germany) Agar medium. Plates were incubated aerobically at 30 °C for 24-48 h. Total yeasts count was enumerated on Potato Dextrose Agar (PDA, Oxoid, UK) at 25 °C for 24-48 h. Total aerobic bacteria was enumerated on Violet Red Bile Glucose (Oxoid, UK) (37°C for 24-48 h). Total Coliform was enumerated on Violet Red Bile Lactose Agar (Oxoid, UK) (37°C for 24-48 h). Kanamycin Aesculin Azide Agar (Oxoid, UK) was used for isolation of Enterococci (37°C/24-48 h). For yeast and moulds, Rose Bengal Chloramphenicol Agar (Oxoid, UK) was used (25°C/4-5 days). Counts for total Staphylococci were carried out on Baird Parker Agar (Oxoid, UK) (37°C / 24-48 h). Typical and atypical Staphylococci colonies were subjected to the Gram stain test, coagulase, catalase test and latex agglutination test (Oxoid, UK).

**Statistical analysis:**

ANOVA was performed to analyse the significance between the means. Results were considered as significant when p values were less than 0.05.

**Results**

Although kefir given in drinking water seemed to slightly improve animal performance by increasing body weight and daily weight gain and by decreasing daily feed intake and feed conversion ratio (FCR) the differences between supplemented groups and the control group were not statistically significant (p>0.05) (Table 2).

The numbers of total bacteria (TAB) cfu were significantly lowered in kefir treated birds compared to the controls (p<0.05 in the group 1 and p<0.01 in the group 2) (figure 1) between 0 and 4 weeks by an average factor of 1 log/cfu in the group 1 (0.20% kefir supplementation) and by average factor of 2 log/cfu in the group 2 (0.50% kefir supplementation) (Table 3). In the same way, faecal coliform (p<0.05 in

![FIGURE 1. — Variations of populations of total aerobic bacteria cfu in faeces of geese supplemented by kefir in drinking water (0.20% in the group 1, 0.50% in the group 2) or not (control group) during 5 weeks.

*Group with an asterisk is statistically significant than the control group.*](image-url)
The effect of a fermented probiotic, on intestinal flora of poultry geese

Groups 1 and 2 (figure 2) and Enterobacteriaceae populations (p<0.05 in the group 2) (figure 3) were significantly reduced in the treated groups. CFUs of Enterococci and yeasts were also diminished compared to the control group but the differences were not statistically significant (figure 4 and 5). The average decreases of micro-organism numerations compared to the control birds were 1.2 log/cfu and 1 log/cfu in the group 1 for coliform and Enterobacteriaceae populations, Enterococci and yeasts respectively and 2 log/cfu, 1.8 log/cfu and 1.2 log/cfu in the group 2 (Table 3). By contrast, the Staphylococci populations were slightly affected by kefir supplementation, particularly between 2nd and the 3rd weeks of treatment: at this time, the CFU numbers were quite similar in the 3 groups (figure 6). The Lactobacilli population was markedly enhanced in kefir treated birds (figure 7). The average percentages of increases were 1 log/cfu and 2 log/cfu in the groups 1 and 2 respectively (Table 3).

Discussion

It has been demonstrated that the most important factor contributing to carcass contamination is intestinal colonization [40]. Faecal matter may contaminate the meat upon

**TABLE 1.** Composition and analysis of diet given to geese (%).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet</th>
</tr>
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<tbody>
<tr>
<td>Corn</td>
<td>61.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>32.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>4.90</td>
</tr>
<tr>
<td>Lime stone</td>
<td>1.20</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.80</td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
</tr>
<tr>
<td>Vit. Min. Premix*</td>
<td>0.35</td>
</tr>
<tr>
<td>Methionin</td>
<td>0.25</td>
</tr>
<tr>
<td>Lysin</td>
<td>0.15</td>
</tr>
<tr>
<td>Chemical analysis (% of dry matter)</td>
<td>89.55</td>
</tr>
<tr>
<td>Metabolisable energy, kcal/kg**</td>
<td>2920</td>
</tr>
<tr>
<td>Crude protein</td>
<td>22.37</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3.77</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>3.61</td>
</tr>
<tr>
<td>Ash</td>
<td>6.14</td>
</tr>
</tbody>
</table>

* Provided per kg concentrate; Vitamin A 21,000 IU; Vitamin D₃ 4,200 IU; Vitamin E 52.5 mg; Vitamin K₃ 8.75 mg; Vitamin B₆ 4.38 mg; Vitamin B₁₂ 8.75 mg; Vitamin B₁ 7 mg; Vitamin B₂ 0.05 mg; Niacin 52.5 mg; Vitamin C 70 mg; Ca-D-Pantothenate 15.75 mg; D-Biotin 0.09 mg; Choline chloride 525 mg; Folinate acid 1.14; Mn 140 mg; Fe 140 mg; Zn 140 mg; Cu 17.5 mg; 1 1.05 mg; Co 0.53 mg; Se 0.44 mg.

** Provided by calculation.

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**Figure 2.** Variations of populations of coliform CFUs in faeces of geese supplemented by kefir in drinking water (0.20% in the group 1, 0.50% in the group 2) or not (control group) during 5 weeks.

*Group with an asterisk is statistically significant than the control group.

**Figure 3.** Variations of populations of Enterobacteriaceae CFUs in faeces of geese supplemented by kefir in drinking water (0.20% in the group 1, 0.50% in the group 2) or not (control group) during 5 weeks.

*Group with an asterisk is statistically significant than the control group.

**Figure 4.** Variations of populations of Enterococci in faeces of geese supplemented by kefir in drinking water (0.20% in the group 1, 0.50% in the group 2) or not (control group) during 5 weeks.

**Figure 5.** Variations of populations of Staphylococci in faeces of geese supplemented by kefir in drinking water (0.20% in the group 1, 0.50% in the group 2) or not (control group) during 5 weeks.

**Figure 6.** Variations of populations of Lactobacilli in faeces of geese supplemented by kefir in drinking water (0.20% in the group 1, 0.50% in the group 2) or not (control group) during 5 weeks.

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slaughter and further processing, resulting in increased public exposure to food borne pathogens. In the processing

### TABLE 1. — Composition and analysis of diet given to geese (%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Group I (0.20% kefir)</th>
<th>Group II (0.50% kefir)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>2550.1 ± 58.7</td>
<td>2572.7 ± 51.2</td>
<td>2585.7 ± 63.4</td>
</tr>
<tr>
<td>ADG (g)</td>
<td>58.3 ± 2.4</td>
<td>58.8 ± 2.3</td>
<td>59.1 ± 2.6</td>
</tr>
<tr>
<td>Daily feed intake (g)</td>
<td>125.3 ± 7.3</td>
<td>122.9 ± 7.1</td>
<td>119.0 ± 6.9</td>
</tr>
<tr>
<td>FCR (g/g)</td>
<td>2.15 ± 0.3</td>
<td>2.09 ± 0.3</td>
<td>2.01 ± 0.2</td>
</tr>
</tbody>
</table>

ADG: Average daily gain - FCR: Feed conversion ratio.

### TABLE 2. — Performance of geese received kefir in drinking water during 5 weeks (0.20% Group I - 0.50% Group 2).

Results are expressed as means ± standard errors.

<table>
<thead>
<tr>
<th>TAB</th>
<th>Coliforms</th>
<th>Enterobacteriaceae</th>
<th>Enterococci</th>
<th>Yeast</th>
<th>Staphylococci</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>7.00*</td>
<td>5.33*</td>
<td>6.32</td>
<td>4.82</td>
<td>4.25</td>
<td>5.25</td>
</tr>
<tr>
<td>Group 2</td>
<td>6.28**</td>
<td>4.54*</td>
<td>5.39*</td>
<td>4.05</td>
<td>4.09</td>
<td>4.90</td>
</tr>
</tbody>
</table>

TAB: Total aerobe bacteria. * (p<0.05), ** (p<0.01) mean significant differences of kefir treated groups compared to control group.

### TABLE 3. — The average percentages for 5 weeks of decrease or increase of a given micro-organism population in geese supplemented by kefir in drinking water (0.20% in the group 1, 0.50% in the group 2) compared to untreated birds (control), determined as means of cfu/g from viable counts.

of poultry, the removal and elimination of such microbial contamination and the prevention of cross-contamination by any potential pathogens have to be the major objectives of hygienic measures. Reducing or eliminating undesirable bacteria in the gastrointestinal tract of poultry can decrease the risk of cross-contamination during evisceration resulting in less contaminated carcasses. Methods that will exclude undesirable bacteria from the animals or reduce their shedding without affecting animal weight gain will be accepted more easily by the livestock producer if they do not result in higher production costs.

Many investigations have been conducted to determine the effect of Lactobacilli cultures on the performance and intestinal microflora of domesticated animals since single or a mixture of Lactobacilli cultures have been found to improve broiler growth. Beneficial effects of Lactobacilli cultures on the growth of chicken were reported by several workers [20, 23, 38, 39, 50]. Furthermore, supplementing the Lactobacilli cultures, singly or in a mixture, in the diet of broilers also decreased significantly (p<0.05) the coliforms in the ileum and caecum [24, 44]. Although it has been reported that the most effective mixture of bacteria for competitive exclusion (CE) is freshly isolated caecal material from healthy adult birds, however the supply of this material is low [22]. As alternatives to fresh caecal bacteria, commercially produced defined and undefined cultures are used. Undefined cultures are reported to be more effective [22] but they risk to transfer opportunistic pathogens to susceptible consumers [33]. Furthermore, complex mixtures of bacteria have been found more effective than single species or simple mixtures [30, 34, 46].

Kefir is a unique natural product containing complex mixtures of lactic acid bacteria (10^8-9 cfu/ml) and yeasts (10^4 cfu/ml) [32]. Numerous species have been associated with the kefir grains and the organisms that have been identified are shown in Table 4 [54]. Fermented milk products such as yoghourt and kefir have been shown to inhibit Salmonella

![Figure 6](image-url)
spp. and *Shigella* spp. growth [1, 41]. Some strains of several Lactobacilli and yeast in kefir grains have already been reported to be probiotic such as *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. plantarum*, *L. brevis*, *L. fermentum*, *L. casei*, *L. helveticus*, *Lac. Lactis* subsp. *lactis*, *Streptococcus thermophilus*, *Saccharomyces cerevisiae* and these are used in probiotic preparations [16].

Water treatment with kefir significantly affected the population numbers of bacteria examined in this study. The main differences between kefir treated birds and controls were observed for total aerobic bacteria and Lactobacilli populations. The counts of TAB were significantly lower in group 1 (p<0.05) and group 2 (p<0.01). Our results were in agreement with the findings of SHOEIB *et al.* [44] but TORTUERO [47], and JIN *et al.*, [24, 25], did not find significant differences in the total aerobies in the small intestine and caecum of broilers fed with or without Lactobacilli culture. Lactobacilli populations were significantly enhanced in geese receiving the highest dose of kefir in drinking water (0.5%) compared to the controls. Likewise, JIN *et al.*, [24] observed a significant (P<0.05) increase of the Lactobacilli populations in the caecum of 10 days-old broilers receiving 0.05 or 0.10% Lactobacilli culture and in the small intestine of broilers receiving 0.15% Lactobacilli culture than the control broilers. However, no significant difference of the numbers of Lactobacilli in the small intestine and caecum of broilers fed with or without Lactobacilli culture was observed when birds were 20, 30 and 40 days-old. In another study of JIN *et al.*, [25], supplementing the diets with a single strain of *L. acidophilus* or a mixture of Lactobacilli did not significantly increase the lactobacilli population in the ileum. In the caecum, however, Lactobacilli populations were significantly higher (p<0.05) than those in the 30 days old control. WATKINS and KRATZER [50, 51] found only slightly higher numbers of Lactobacilli in the intestine of chicks dosed with host specific Lactobacilli strains. These conflicted results reported for Lactobacilli populations in broiler intestines may be resulted from the heterogeneity of the numbers of supplied germs through probiotic mixture, or from the density of populations of the other intestine and caecal bacteria. The other important factor may be the viability of the probiotic preparation: indeed, the viability of the preparation is not always checked before it is used and it is important to select strains with maximum ability to survive in the intestine, maximum epithelial adhesion and growth rate. Diet is another factor which may be influencing the results obtained with probiotics.

Colony forming units of *Enterobacteriaceae*, often associated with intestinal disease, were significantly lower in group 2 (0.50% kefir supplementation), indicating a Lactobacilli - *Enterobacteriaceae* antagonism, which has been already proposed by JUVEN *et al.*, [26] and REID *et al.*, [42]. Moreover, KARAMAN *et al.*, [27] also reported a reduction of the *Enterobacteriaceae* population in broilers fed diets with organic acid+yeast (*Acid Lac Dry*+*Yea Sacc 1026*) supplementation. By contrast, VAHJEN *et al.*, [48] did not detect any difference in the numbers of *Enterobacteriaceae* in their study in which, they used a probiotic strain of *Enterococcus facei* in the small intestine of growing turkey poults. However, SHOEIB *et al.*, [44] also reported reduced *E. coli* counts in chickens after 2 weeks of treatment with a commercial probiotic ‘Pronifer’ containing viable lactic acid bacteria. Likewise, treatments of chickens with *L. acidophilus* and *S. faecium* against *C. jejuni* colonization resulted in 70% fewer chickens shedding *C. jejuni* when compared with control chickens over a period of 40 days. Overall, the number of chickens shedding *C. jejuni* in the intestinal tract was significantly higher in the control group [37]. *C. jejuni* has often been responsible for human gastroenteritis and poultry has been implicated as a source of these human infections as intestinal colonization of *C. jejuni* in the chicken plays a role in carcass contamination during slaughter. Lactobacilli cultures (*L. acidophilus*, *L. fermentum*, *L. crispatus* and *L. brevis*) have been shown to have an antagonistic effect on *C. jejuni* in a stimulated chicken digestive tract model [7]. Consequently, the reduction of *C. jejuni* colonization in chickens using probiotics can potentially reduce the incidence of *C. jejuni* infections in humans. However, field trials pose several difficulties that are not experimented in laboratory studies, such as there can be no proper control of flock. BLANKENSHIP *et al.*, [5] used an undefined culture by a two-stage treatment of broiler chicks in which spray-inoculation of chicks in the hatchery was followed by drinking-water administration on the farm. The effect of treatment against salmonella was significant (p<0.05), and after processing, the prevalence of *Salmonella* contaminated carcasses was only 10% for the treated flocks compared with 41% for the untreated controls (P<0.05). However, GOREN *et al.*, [19] could observe no ultimate benefit from CE treatment because of contamination during transportation of chickens to the processing plant and subsequent processing. Nevertheless, there is some evidence that even an existing infection can be reduced under field conditions with a CE preparation [6].

In this study, a significant (p<0.05) decrease of the coliform population was observed in the faeces of goslings supplemented with kefir Lactobacilli-yeast during the whole experimental period. These results are in agreement with SHOEIB *et al.*, [44], TORTUERO [47] and WATKINS and KRATZER [50], who reported that chicks treated with Lactobacilli strains, had lower numbers of coliforms in caecal samples than the controls. Similar results have also been reported by FRANCIS *et al.*, [15] in the caecum and small intestine of turkeys. Furthermore, the Lactobacilli strains used in the study of JIN *et al.*, [23] have been found to be able to inhibit the growth of three serotypes of *E. coli* O1:K1, O2:K1, and O78:K80 in vitro. However, JIN *et al.*, [24] reported significant reductions of coliform populations in the caecum of 10 days old broilers fed with diets containing (0.05, 0.10 and 0.15 %) of Lactobacilli spp. culture. In the same way, broilers receiving 0.05 % Lactobacilli culture at 20 and 30 days of age and 0.10 % Lactobacilli culture at 20 days of age also presented reduced coliform populations. By contrast, no difference for coliform population was observed in the caecum of broilers fed with diets enriched by Lactobacilli culture at 40 days of age. In another study of JIN *et al.*, [25], although there was no significant difference between the coliform populations in the ileum of broilers recei-
ving or not Lactobacilli cultures, broilers supplemented with Lactobacilli cultures had slight lower numbers of coliforms in the ileum, except at 10 days of age. The addition of either L. acidophilus or a mixture of 12 strains of Lactobacilli isolates (2 strains of L. acidophilus, 3 strains of L. fermentum, 1 strain of L. crispatus and 6 strains of L. brevis) in the diet decreased significantly (P<0.05) the numbers of coliforms in the caecum 10 and 20 days after feeding, but there was no differences at 30 and 40 days of age.

Previous studies indicated that probiotic preparations added to animal feed may improve the feed conversion ratio, the growth rate, or may prevent diarrhoea or colonization by food borne pathogens. Many feeding trials have shown significant variations [6, 9, 12, 17, 20, 23]. These differences might be resulted from inter-individual variability [45, 49]. This indicates that the intestinal microflora of each individual animal may react differently to the presence of probiotics, and the influence of the probiotic strain may diminish when the animals were older. In this study, addition of kefir into drinking water did not significantly improve growth rate and feed conversion ratio in growing goslings (p<0.05).

More recently, there has been an interest for enhancing the protective efficiency of undefined CE preparations by the supplementary administration of lactose or other dietary carbohydrates [10, 11, 12, 43]. After milk turning into kefir beverage, lactose and its constituents, galactose and glucose are present and in kefir, and they may provide an advantage since in the study of HINTON et al., [21], the most effective protection (a reduction of 5.5 log_{10}) in caecal carriage, was achieved from the combined use of lactose and a protective caecal culture. Likewise, the results obtained in the study of YURTALAN and ATES [55] were in agreement with the findings of other researchers [10, 21].

Overall, the present study indicated that addition of kefir as Lactobacilli-yeast supplement in the diets of goslings increased significantly the numbers of lactobacilli which is considered to provide balanced microflora of intestine; decreased significantly the numbers of total aerobic bacteria, coliforms, Enterobacteriaceae, and Enterococci The results of this study provided a preliminary information that kefir Lactobacilli-yeast may be a useful candidate as a CE preparation to improve intestinal microflora of poultry. Further studies should consider the evaluation of microbial populations associated with poultry carcasses to determine whether such treatment could be developed as a practical intervention method for reducing microbial load of carcasses and human exposure to food borne pathogens particularly Campylobacter spp., Salmonella spp. which are the challenging organisms in human nutrition.

References


