DOI 10.1515/JAS-2016-0023 J. APIC. SCI. VOL. 60 NO. 2 2016

DE GRUYTER OPEN

Original Article

EFFECTS OF ROYAL JELLY AND BEE POLLEN ON THE GROWTH OF SELECTED PROBIOTIC BACTERIA (*BF. ANIMALIS* SPP. *LACTIS, L. ACIDOPHILUS AND L. CASEI*)

Metin Guldas
Uludag University, Karacabey Vocational School, Department of Food Processing Karacabey 16700 Uludag University, Bee Keeping Development, Application and Research Center, Gorukle Campus Nilufer 16059 Bursa, Turkey

corresponding author: metin.guldas@gmail.com
Received: 11 April 2016; accepted: 6 October 2016

Abstract

In this research article, the effects of bee pollen and royal jelly on the selected probiotic bacteria, as growth factors, were investigated. The probiotic cultures were activated in MRS broth at 37°C. Then, bee pollen and royal jelly (10 mg/100 µL, 25 mg/250 µL, 50 mg/500 µL, 75 mg/750 µL, and 100 mg/1000 µL) were added on the probiotic cultures in MRS broth and sampled at 0, 24, and 48 hours of incubation. The medias used for enumeration of the probiotic cultures were RCA (Reinforced Clostridial Agar) for *Bf. animalis* spp. *lactis*, MRS (deMann, Rogosa and Sharpe) Agar with D-sorbitol for *Lb. acidophilus* and MRS-Vancomycine Agar for *Lb. casei*. The lactic acid production by *Lb. acidophilus*, *Lb. casei*, and *Bf. animalis* spp. *lactis*, and acetic acid production by *Bf. animalis* spp. l*actis*, were determined to compare the bacterial proliferation. The probiotic cultures were mainly affected by the bee pollen and royal jelly during the first 24 hours. The changes observed in the number of probiotic counts between 24 and 48 hours were not significant, statistically ($P<0.05$). Generally, the probiotic bacterial counts increased parallel to the concentration of bee pollen or royal jelly up to 75mg, and remained unchanged above this concentration. In terms of lactic acid production and bacterial growth, the most significant growth was observed on *Lb. acidophilus* when bee pollen or royal jelly was added.

Keywords: bee pollen, *Bifidobacterium animalis* spp. *lactis, Lactobacillus acidophilus*, *Lactobacillus casei*, royal jelly

INTRODUCTION

Royal jelly is a bee product secreted by the hypopharyngeal glands of young worker (nurse) bees, to feed young larva and the adult queen bee. This jelly is a thick, water-soluble and viscous product. Royal jelly contains many proteins and free amino acids, 18-52% total sugar, free fatty acids, as well as water soluble vitamins, minerals, and enzymes. It has been mentioned that royal jelly has antibiotic activity due to high concentration of l0-hydroxydecanoic acid in the jelly (FAO, 2013a). Antibiotic effects of royal jelly have been determined against the pathogenic bacteria such as *Salmonella, Escherichia coli, Micrococcus pyrogens, Proteus, Staphylococcus aureus*, and *Bacillus subtilis* (Yatsunami & Echigo, 1985; Bărnuțiu et al., 2011; FAO, 2013a). In addition to antimicrobial properties, honey may contain several pathogenic microorganisms. For this reason, honey can be called a reservoir for microorganisms (Olaitan et. al., 2007). *Clostridium botulinum* is a significant risk factor for infants. The health problem caused by this microorganism is called infant botulism. Infant botulism is a toxigenic infection which is harmful for infants under the age of one year (Rudnicka et al., 2015).

Pollen is a natural product which consists of the grain particles of the male gametophyte. These particles are gathered and produced by bees from flowering plants. Pollen as a food source for bees is rich in nutrients such as proteins and amino acids, lipids, sugars (fructose, glucose, and sucrose), vitamins (B, C, and E), minerals (Ca, Mg, and P), trace elements (Fe, Cu, Mn, and Zn), hormone-like growth factors (auxins and gibberellin) as well as phytochemicals such as phytos-

terols, carotenoids, and flavonoids (Kacainova et al., 2012; Komosinska-Vassev et al., 2015; FAO, 2013b).

In recent years, consumption of probiotic foods has increased due to the many health benefits such as reducing serum cholesterol level, improving lactose utilisation to prevent lactose intolerance, preventing gastrointestinal cancers, keeping intestinal microflora active, and supporting immune system. Probiotics prevent harmful pathogenic bacteria in the human body and help to control intestinal infections due to producing antimicrobial compounds such as hydrogen peroxide, antibiotics, deconjugated bile acids, and organic acids (Goderska & Czarneski, 2007; Baroutkoub et al., 2010; Kabeerdoss et al., 2011; Wang et al., 2012; Shori & Baba, 2014). The microorganisms named as probiotics are the subgroup bacteria belonging to genus of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Saccharomyces*, *Aspergillus*, and *Torulopsis*. Due to organic acids (lactic, and acetic acids e.g.) produced by lactic acid bacteria, the probiotics lower pH and prevent the proliferation of pathogenic and deteriorative bacteria (Simsek et al., 2002; Strus et al., 2004; Valdez et al., 2005; FAO/WHO 2006).

In the literature, it has been found that the effects of honey on the probiotic bacteria (Ustunol & Gandhi, 2001; Shin & Ustunol, 2005) and yoghurt bacteria (Varga, 2006) were sufficiently investigated. But, the effects of pollen and royal jelly on the growth of probiotic bacteria have not been studied in detail (Nabas et al., 2014; Yerlikaya, 2014). Different results have been found due to the materials and methods used, and the growth environment tested. The analytical and microbiological results can differ according to the chemical and microbiological characteristics of yoghurt. The high acidity of the yoghurt and other inhibitory factors available in milk such as antibacterial peptides liberated from the certain milk proteins (Fadaei, 2012; Vajiheh, 2012), can interfere to obtain actual test results.

There are a number of factors that can affect the growth of *Bifidobacterium* spp. in milk products such as strains of probiotic bacteria, pH, the presence of organic acids, interactions with other microorganisms, storage temperature, and production conditions (Shah, 2000; Boylston et al., 2004). Shin & Ustunol (2005) stated that the growth of *Bifidobacterium* spp. was enhanced by the addition of honey while *C. perfringens* and *E. aerofaciens* were inhibited if they co-cultured with *Bifidobacteria*. Bee pollen also contains a number of microorganisms which come from nature (Brindza et al., 2010).

The most significant issue for the probiotic food products is to contain enough viable probiotic bacteria at the moment of consumption (10^8-10^9 log cfu ml^{-1}). Probiotic foods that contain a low number of viable probiotic organisms do not provide expected health benefits for the consumers. Therefore, such prebiotic compounds as inulin and fructooligosaccharides (FOS) have been added to probiotic foods to reach desired viable counts. Bee pollen and royal jelly have known to contain rich nutrients for the human diet. But, on the other hand, even bee pollen or royal jelly also contains some antimicrobial compounds.

In this research, the effects of bee pollen and royal jelly containing a number of nutrients, on the growth of three probiotic bacteria cultivated in the selective media, were investigated.

MATERIALS AND METHODS

Probiotic cultures

The probiotic cultures used in the research were *Bifidobacterium animalis spp. lactis* (BB12, DSM15954), *Lactobacillus acidophilus* (LA-5, DSM13241) and *Lactobacillus casei* (431, ATCC55544) obtained from Chr. Hansen Co., Horsholm, Denmark. Ten grams of the probiotic cultures were weighed, transferred into 20 mL sterilised water and shaken properly to obtain a homogenised culture. Each culture suspension (20 mL) was added and activated in 50 mL of MRS (deMann, Rogosa and Sharpe) broth containing 5 % (w/v) lactose at 37°C for 24 h to obtain an approximately 108 cfu/mL bacterial count (Popa & Ustunol, 2011). The reactivation procedure and the estimation were

repeated on the same culture media (MRS broth) in the above-mentioned conditions for specified probiotic bacteria, until the targeted initial bacterial count (approx. 108 cfu/mL) was obtained as described by Guldas & Irkin (2010). Then, 1 mL of each probiotic bacteria was transferred into 9 mL of MRS broth without pollen and royal jelly to act as the control. Only *Bifidobacterium animalis spp. lactis* was grown and incubated in MRS broth at 37°C for 24 hours, under anaerobic conditions using GasPacks (BBL Microbiology Systems, Cockeysville, MD, USA).

Sample preparation

The pure royal jelly and bee pollen samples were supplied from the Uludag University Bee Keeping Development, Application and Research Centre (AGAM, Bursa, Turkey). The samples were kept in a refrigerator at 4°C. The bee pollen samples were collected by pollen traps. The pollen samples were provided by AGAM in the first half of May, 2015. The botanical origins (plant taxa) of pollens identified in the same rural area at the same seasonal time were given as follows (Bilisik et al. 2008): *Brassicaceae* (32.70 %), *Salix* (29.35 %), *Rosaceae* (21.05 %), *Papaver* (10.37 %), *Pinus* (1.91 %), *Cichorioideae* (1.75 %), *Trifolium pratense* (1.59 %), *Acer* (0.64 %), and *Boraginaceae* (0.64 %).

The bee pollen and royal jelly samples were prepared for analyses in a laminar flow cabinet (ESCO, Class II, Germany) to avoid contamination. The bee pollen and the royal jelly samples were dissolved in sterilised water at room temperature and 35°C and obtained 10 % of the bee pollen and royal jelly solutions, respectively. From these solutions, 100 µL, 250 µL, 500 µL, 750 µL, and 1000 µL of the samples were pipetted into the MRS broth with lactose (5 % w/v). Therefore, concentrations of the bee pollen and royal jelly added into the MRSL broth tubes were 10 mg / 100 µL, 25 mg / 250 µL, 50 mg / 500 µL, 75 mg / 750 µL, and 100 mg/1000 µL.

Media and growth conditions

For the bacterial enumeration, 1 mL from each test tube containing MRSL broth was taken and used to prepare serial dilutions before transferring into the petri plates. These petri plates contained the selective media as described below.

Lactobacillus acidophilus was enumerated selectively in MRS (deMann, Rogosa and Sharpe) D-sorbitol (10 g/100 mL) media (Tharmaraj and Shah, 2003) at 37°C for 72 h. The selective enumeration of *Bf. animalis spp. lactis* was implemented in RCA (reinforced clostridial agar) with 0.03 g/100 g aniline blue and dicloxacillin (2 mg/mL, Sigma). The plates were incubated under anaerobic conditions at 37°C for 48 h using GasPacks (BBL Microbiology Systems, Cockeysville, MD, USA) according to Kailasapathy et al. (2008). For enumeration of Lb. casei, MRS-Vancomycine agar was used. The preparation of the MRS Vancomycine agar was done by adding 2 ml of 0.05 g vancomycine (Sigma)/100 ml solution into 1 L of MRS broth to obtain 1 mg/L of the final concentration.

The average count of the duplicate plates or tubes was used for statistical evaluations.

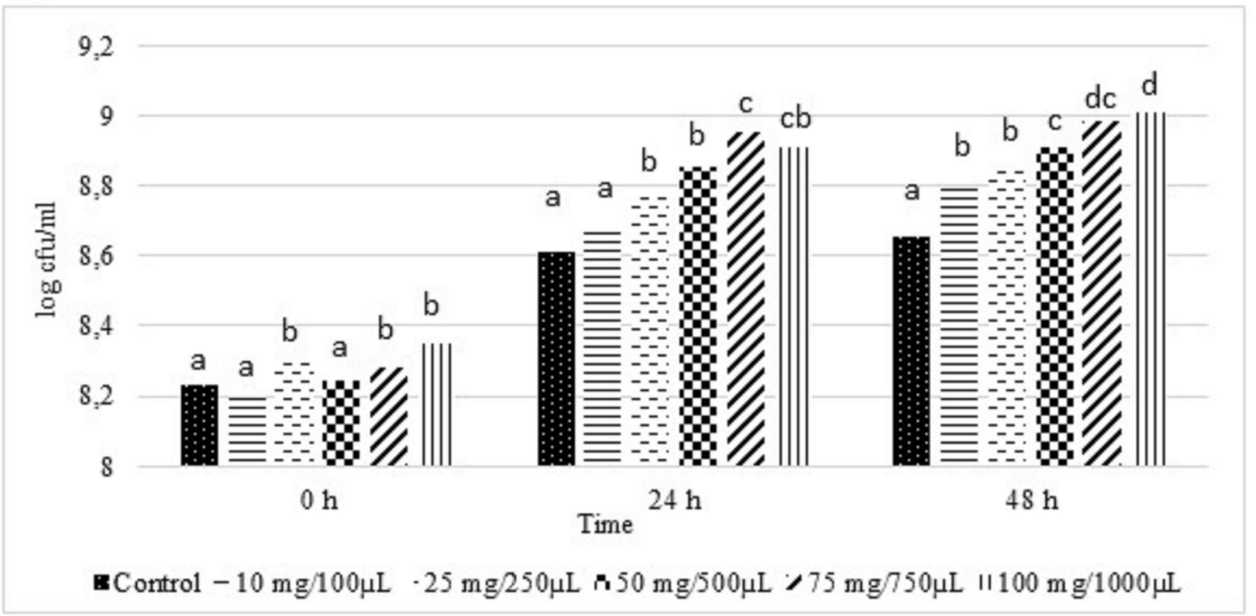
Determination of lactic and acetic acids

Lactic (D-/L-Lactic acids) and acetic acid productions by the tested probiotic bacteria were determined during 0, 24, and 48 hours of incubation in MRS broth. For lactic and acetic acid determinations, the enzymatic method proposed by Popa & Ustunol (2011) was used. Lactic acid productions by *L. acidophilus* (LA-5, DSM13241) and *L. casei* (431, ATCC55544); acetic acid and lactic acid productions by *Bifidobacterium animalis spp. lactis* (BB12, DSM15954) were determined at 340 nm by UV-VIS spectrophotometer (Shimadzu UV-1800, double beam, Japan) using test kits in terms of D- / L- lactic and acetic acid (R-Biopharm Inc., Marshall, MI, USA). The defined acids were determined as g/L.

Statistical analysis

The statistical analyses were done by using SPSS 15.0 software for windows (SPSS Inc., Chicago, Illinois, USA). A one-way analysis of variance (ANOVA) test was used to determine the mean differences. Tukey HSD test was achieved to determine the level of significance between the means.

Fig. 1a.



*Means with different letters on the columns are significantly different ($P < 0.05$), n=3

Fig. 1a. Effect of bee pollen on the growth of **Bf. animalis* spp. lactis*

RESULTS

The analysis of the results presented on Figures 1 and 2, in which the effects of royal jelly and bee pollen on the bacterial growth are reflected, showed that bee pollen or royal jelly exhibited the strongest effect on probiotic bacterial proliferation at the end of 24 h. The number of *Bf. animalis spp. lactis, Lb. acidophilus ve Lb. casei* during the mentioned period was between 8.4-8.8, 8.6-9.0 and 8.4-8.9 log cfu/ml with the addition of the royal jelly; 8.6-9.0, 9.4-9.8, and 8.6-9.0 log cfu/ml with the addition of the bee pollen, respectively.

The highest counts of *Bf. animalis spp. lactis* were obtained in the samples containing 75 mg/750 µL of bee pollen. During the 24 and 48 hours of cultivation, *Bf. animalis spp. lactis* counts increased 8.09 and 8.45%, while the counts regarding the same probiotic bacteria increased 4.61 and 5.10% in the control group, respectively (Fig. 1a).

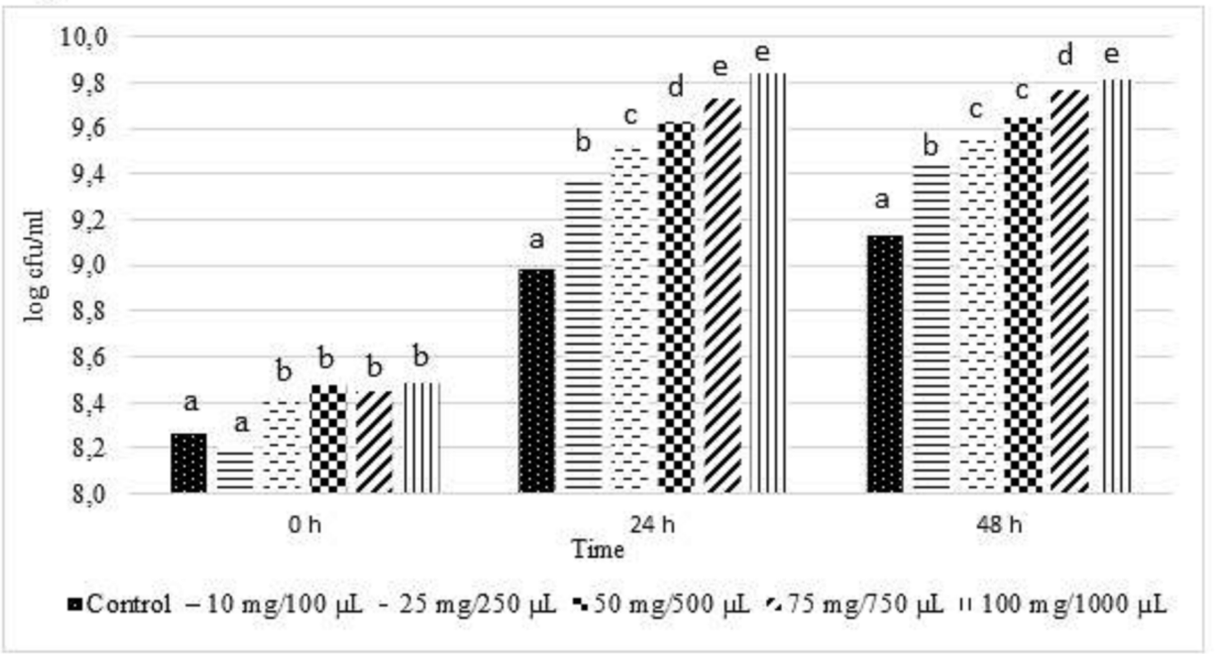
While the number of *Lb. acidophilus* in the control group increased 8.70% at the end of 24 hours, it increased 13.3-15.9% in the bee-pollen-added samples parallel to the elevated concentration. The increase in the same bacteria was 13.8-15.6% after the bee pollen addition at the end of 48 hours. As seen from Figure 1b, there was no difference statistically ($P<0.05$) in *Lb. acidophilus* counts between 24 and 48 hours of cultivation, after the addition of bee pollen.

The highest increase in the *Lb. casei* counts was approximately 10% and obtained from the samples which had an addition of 75 mg/750 µL of bee pollen (Fig. 1c). The number of *Lb. casei* did not change at the end of 48 hours nor did the concentration elevate to 100 mg/1000µL of bee pollen ($P<0.05$). The number of *Lb. casei* neither changed at the end of 48 hours nor did the bee pollen concentration was elevated up to 100 mg/1000µL ($P<0.05$).

The increase in *Bf. animalis spp. lactis* counts depended on the royal jelly concentration (Fig. 2a). *Bifidobacterium animalis spp. lactis* increased approximately 7.5% in the samples which had 75 mg/750 µL of royal jelly added at the end of 24 hours. However, neither 75 mg/750 µL nor 100 mg/1000 µL of royal jelly caused a significant difference in the *Bf. animalis spp. lactis* counts ($P<0.05$).

The addition of royal jelly led to the highest *Lb. acidophilus* levels from among all the investigated probiotic bacteria (Fig. 2b). The number

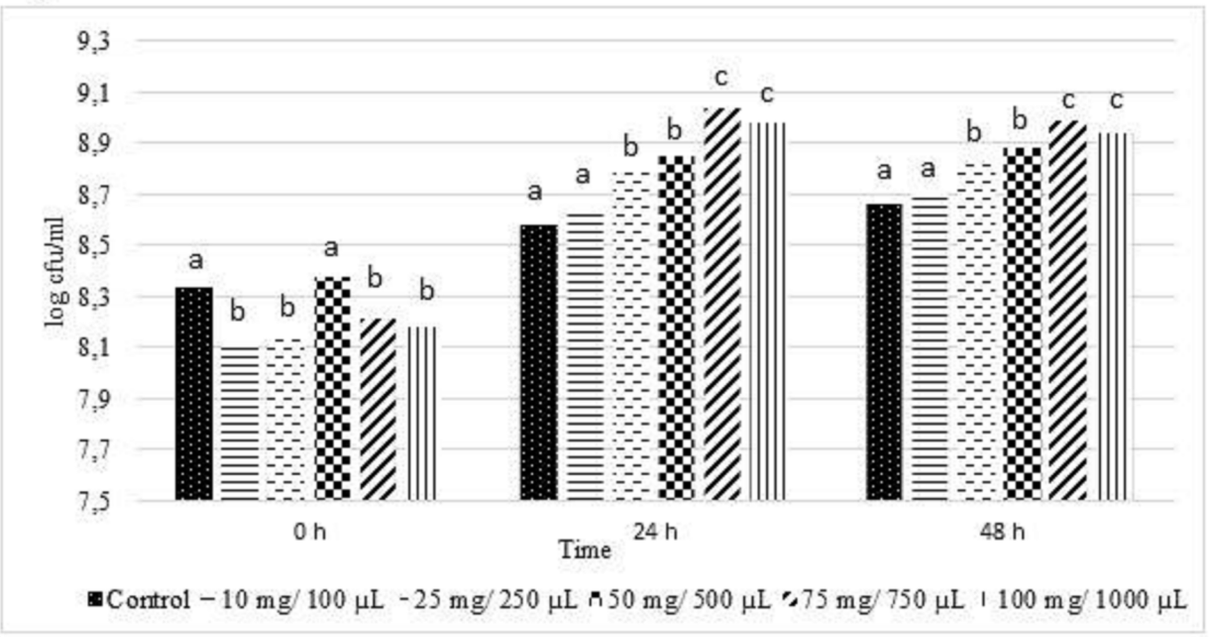
Fig. 1b.



*Means with different letters on the columns are significantly different (P < 0.05), n=3

Fig. 1b. Effect of bee pollen on the growth of **Lb. acidophilus**

Fig. 1c.



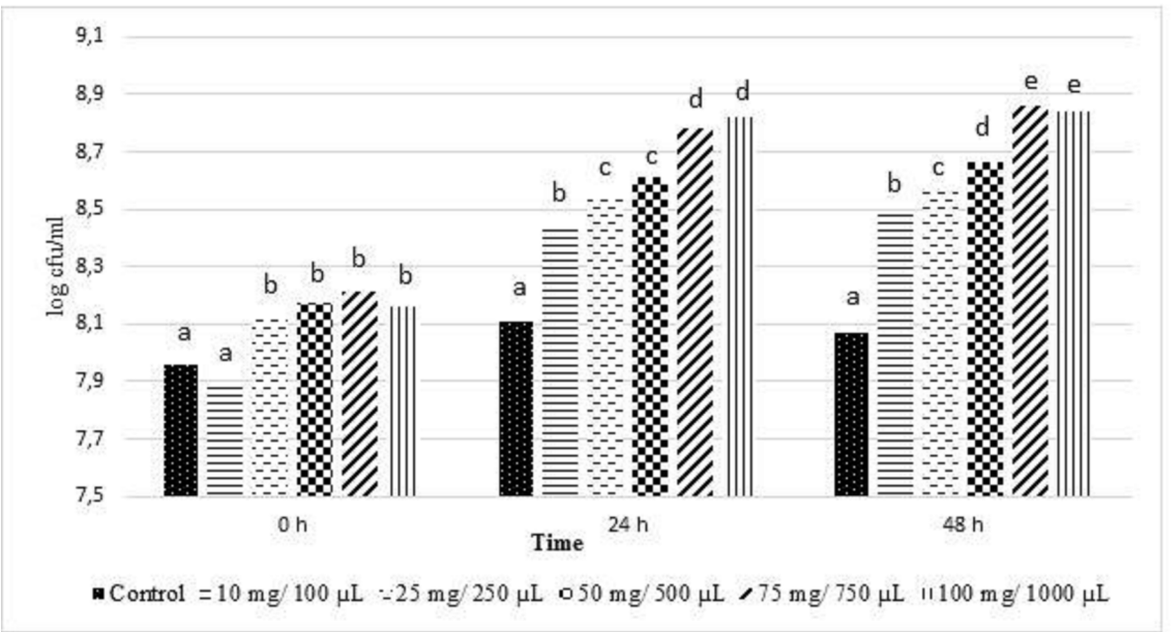
Means with different letters on the columns are significantly different (P < 0.05), n=3

Fig. 1c. Effect of bee pollen on the growth of **Lb. casei**

of *Lb. acidophilus* reached the highest level (9.81 log cfu/ml) after 24 hours, in the samples in which 75 mg/750 µL of royal jelly had been added. *Lactobacillus casei* counts in the control group in which no royal jelly had been added, increased 1.46 and 2.19%. *Lactobacillus casei* counts increased 8.42 and 9.52% in the samples in which 75 mg/750 µL of royal jelly had been added after 24 and 48 hours, respectively (Fig. 2c).

The number of probiotic bacteria in the samples in which bee pollen was added, increased or

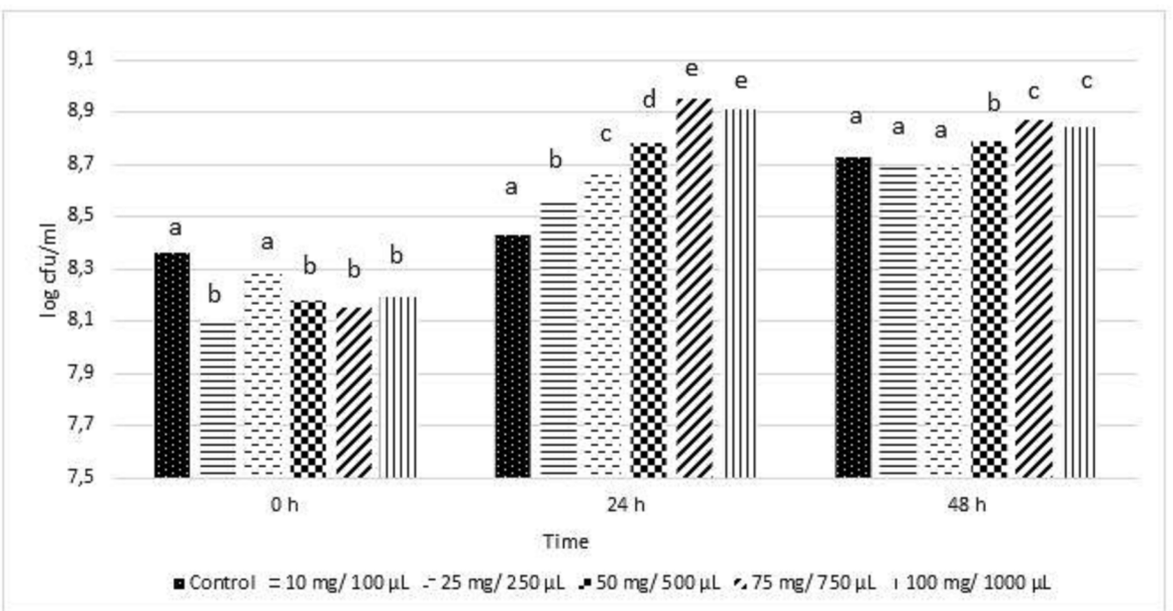
Fig. 2a.



*Means with different letters on the columns are significantly different ($P < 0.05$), n=3

Fig. 2a. Effect of royal jelly on the growth of **Bf. animalis* spp. *lactis**

Fig. 2b.

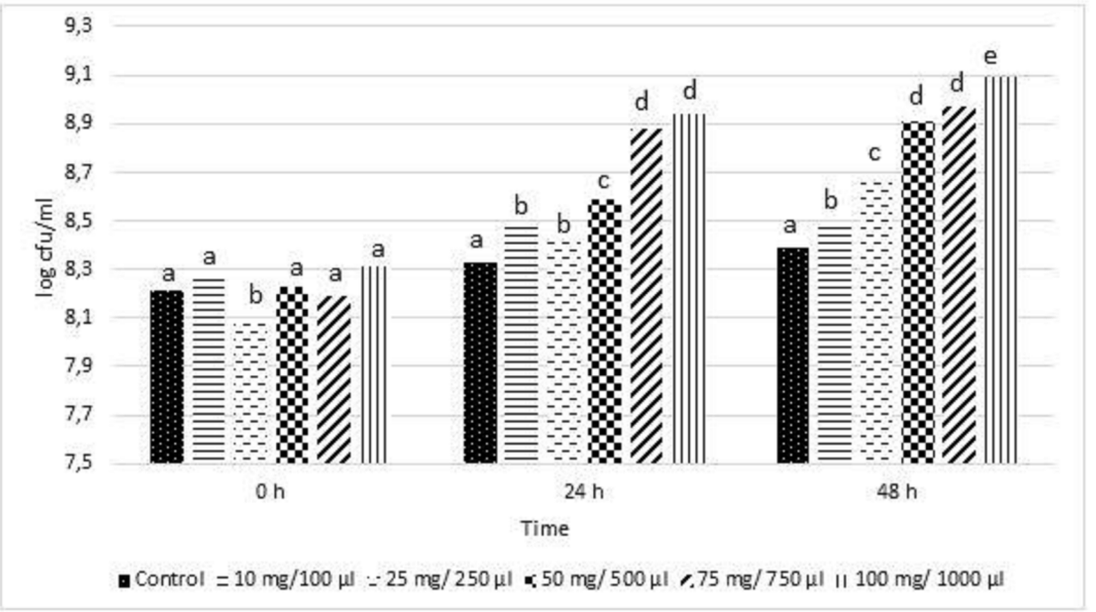


*Means with different letters on the columns are significantly different ($P < 0.05$), n=3

Fig. 2b. Effect of royal jelly on the growth of **Lb. acidophilus**

decreased slightly between 24 and 48 hours of incubation. But, these changes observed in the probiotic counts were not significant, statistically ($P<0.05$). The concentration in which the maximum observed probiotic growth was 75 mg/750 µL and counts of probiotic bacteria (*Bf. animalis spp. lactis, Lb. acidophilus* and *Lb. casei*) increased only 0.36, 0.50, and 0.60% between 24 and 48 hours in this concentration, respectively. In other words, the elevation of the concentration above 75 mg/750 µL even for bee pollen or royal jelly, did

Fig. 2c.



*Means with different letters on the columns are significantly different ($P < 0.05$), n=3

Fig. 2c. Effect of royal jelly on the growth of **Lb. casei**

not cause a significant increase in the number of the probiotic bacteria (P<0.05)

The highest growth among the investigated probiotic bacteria was observed in *Lb. acidophilus* when there was an addition of bee pollen or royal jelly. When 75 mg/750 µL concentration was considered, the increase in *Lb. acidophilus* counts was approximately 15 and 10% when using bee pollen and royal jelly, respectively.

As seen from the tables, the organic acid productions of the investigated probiotics were parallel to the bacterial growth (Tab. 1, 2, 3, and 4). Even the lactic acid or acetic acid productions increased when there were elevated concentrations of bee pollen and royal jelly. Secondly, organic acid productions and growth stimulation when the bee pollen was used, were higher than the royal jelly addition. During the fermentation of food products, organic acid production is one of the main criterions for monitoring the microbial growth. Lactic acid in fermented milk products is not only the significant indicator of bacterial growth (Bouzas et al., 1991) but also one of most significant taste parameters (Popa & Ustunol, 2011). The lactic acid content of the control tubes ranged between 2.88 and 3.81g/L in my research. As a comparison, Popa & Ustunol (2011) found the lactic acid produced by the bifidobacteria and *Lb. acidophilus* to be 3.08 and 3.59 g/L in the control tubes that contained MRSL broth, respectively.

The lactic acids produced by *Bf. bifidum* and *Lb. acidophilus* in the control tubes were measured as 23.69 and 23.56 g/L at 24 hours of incubation, respectively, in my experiment. As a comparison, Popa & Ustunol (2011) found the production of the lactic acids to be 28.32 and 28.28 g/L in the same growth media (MRSL broth) and at 24 hours of incubation, respectively.

As seen from Table 1, 2, and 3, a distinct increase in the production of the organic acids was observed when comparing to the control groups. The changes observed in the lactic acid (D-/L- Lactic acids) contents between these groups were statistically significant (P≤0.05). Most of the changes in the organic acid contents between 24 and 48 hours of incubation were not statistically significant, (P≤0.05). The differences between the pronounced periods was approximately equal to 1 g/L or lower in terms of the production of the organic acids (Tab. 1, 2, 3, and 4).

In addition, among the three probiotic bacteria, lactic acid produced by *Lb. acidophilus* was slightly higher (Tab. 2) than the other two probiotics (Tab. 1 and 3). This finding can also

Table 1

Effect of bee pollen and royal jelly on production of D-/L-Lactic acids by *Bf. animalis* spp. *lactis**

	0 h	24 h	48 h	0 h	24 h	48 h
The Control	3.11±0.17a	23.69±0.08a	23.45±0.29a	3.11±0.17a	23.69±0.08a	23.45±0.29a
		Bee Pollen			Royal Jelly	
10 mg/ 100 µL	3.72±0.39b	24.91±0.34b	25.20±0.36b	3.28±0.28b	23.55±0.45a	23.18±0.35a
25 mg/ 250 µL	3.34±0.34c	26.67±0.23c	26.33±0.17c	3.25±0.31b	26.19±0.41b	25.89±0.51b
50 mg/ 500 µL	3.81±0.21b	25.46±0.59b	25.92±0.41b	3.37±0.11c	24.88±0.21a	25.07±0.22b
75 mg/ 750 µL	3.25±0.26c	28.28±0.14d	28.66±0.04d	3.64±0.36d	28.77±0.62c	28.44±0.07c
100mg/ 1000 µL	3.46±0.07c	27.71±0.18d	27.54±0.25d	3.42±0.03c	28.64±0.18c	28.15±0.23c

*Means with the same superscripts in the same column are not significantly different (P≤0.05), n=3

Table 2

Effect of bee pollen and royal jelly on production of D-/L-Lactic acids by *Lb. acidophilus**

	0 h	24 h	48 h	0 h	24 h	48 h
The Control	3.27±0.33a	23.56±0.08a	23.86±0.04a	3.27±0.33a	23.56±0.08a	23.86±0.04a
		Bee Pollen			Royal Jelly	
10 mg/ 100 µL	2.88±0.51b	24.28±0.41b	24.75±0.39b	3.16±0.37a	23.33±0.23a	23.48±0.52a
25 mg/ 250 µL	3.03±0.02a	26.41±0.47c	26.73±0.55c	3.09±0.06b	26.09±0.19b	26.72±0.29b
50 mg/ 500 µL	2.94±0.24b	27.17±0.12d	27.65±0.26c	3.02±0.32b	26.19±0.21b	26.38±0.06b
75 mg/ 750 µL	3.20±0.35a	28.45±0.05e	28.76±0.08d	3.42±0.48c	27.87±0.07c	27.13±0.32c
100mg/ 1000 µL	3.07±0.18a	28.22±0.42e	28.54±0.44d	3.75±0.04d	27.95±0.33c	27.10±0.05c

*Means with the same superscripts in the same column are not significantly different (P≤0.05), n=3

be confirmed by the microbial growth of the same bacteria, as can be seen in Figure 1b and 2b. The lowest amount of lactic acid was produced by *Lb. casei.* The production of lactic acids in the tubes containing bee pollen and royal jelly ranged between 23-28 g/L produced by *Bf. bifidum* and *Lb. acidophilus* (Tab. 1 and 2) at 24 or 48 hours of incubation. While the same organic acid content produced by *Lb. casei* was between 21.53 and 23.89 g/L (Tab. 3).

Acetic acid production by *Bf. bifidum* in the control tubes was 0.94 and 1.03 g/L at 0 and

Table 3

Effect of bee pollen and royal jelly on production of D-/L-Lactic acids by *Lb. casei*

	0 h	24 h	48 h	0 h	24 h	48 h
The Control	3.38±0.15a	21.18±0.56a	21.58±0.31a	3.38±0.15a	21.18±0.56a	21.58±0.31a
	Bee Pollen			Royal Jelly		
10 mg/ 100 µL	3.23±0.04a	23.09±0.05b	23.16±0.33b	3.56±0.35a	22.46±0.07b	22.77±0.20b
25 mg/ 250 µL	3.41±0.28a	22.91±0.11c	22.82±0.15b	3.05±0.14b	21.53±0.29a	21.81±0.26a
50 mg/ 500 µL	3.15±0.34a	22.74±0.46c	22.67±0.28b	3.45±0.48a	21.56±0.44a	21.13±0.13a
75 mg/ 750 µL	3.60±0.03b	23.78±0.27d	23.86±0.42c	3.17±0.07b	22.43±0.32b	22.27±0.37b
100mg/ 1000 µL	3.19±0.47a	23.89±0.33d	23.57±0.06c	3.29±0.16b	22.95±0.05b	22.64±0.51b

*Means with the same superscripts in the same column are not significantly different (P≤0.05); n=3

Table 4

Effect of bee pollen and royal jelly on acetic acid production by *Bf. animalis* spp. *lactis**

	0 h	24 h	48 h	0 h	24 h	48 h
Control	0.94±0.03a	1.03±0.35a	1.11±0.13a	0.94±0.03a	1.03±0.35a	1.09±0.13a
	Bee Pollen			Royal Jelly		
10 mg/ 100 µL	0.69±0.26b	0.85±0.09b	1.21±0.16b	0.77±0.07b	0.92±0.20b	1.13±0.09a
25 mg/ 250 µL	0.87±0.11a	1.17±0.06a	1.08±0.47a	1.02±0.10a	0.87±0.25b	0.94±0.22b
50 mg/ 500 µL	1.09±0.48a	1.22±0.17a	1.32±0.29c	0.96±0.08a	1.19±0.06c	1.05±0.41a
75 mg/ 750 µL	1.13±0.21b	1.46±0.43c	1.35±0.26c	1.11±0.05a	1.22±0.33c	1.17±0.19a
100mg/ 1000 µL	0.92±0.35a	1.38±0.33c	1.27±0.23c	1.04±0.28a	1.23±0.08c	1.26±0.09c

*Means with the same superscripts in the same column are not significantly different (P≤0.05), n=3

24 hours of incubation, respectively (Tab. 4). In the findings of Popa & Ustunol (2011), the acetic acid content was slightly higher: 1.16 and 1.34 g/L, in the control tubes in the same measurement periods.

DISCUSSION

The increase observed in the probiotic bacterial counts during the first 24 hours, and following stationary growth in the later 24 hours, is probably related to the consumption rate of

the added nutrients by the probiotic bacteria. The majority of bee pollen and royal jelly which are rich in terms of nutritious compounds were probably consumed in the first 24 hours by the probiotic bacteria. Even royal jelly or bee pollen has rich nutrient contents. Most of them are of simple and small molecules to stimulate microbial growth. Probably, the chemical composition of the bee pollen and royal jelly caused a synergistic effect on the probiotic bacterial growth. The chemical composition available in the dairy products is very important in terms of metabolic activity of probiotic bacteria. Types and quantities of such nutrients as carbohydrates, peptides, and amino acids are significant factors in terms of the synergistic effect (Dave & Shah, 1998; Heller, 2001; Vajiheh, 2012).

On the other hand, bee products also contain several inhibitor components affecting microbial growth (Kacainova et al., 2012). The positive results concerning the symbiotic effects of royal jelly with *L. acidophilus* and *Bf. animalis* spp. *lactis* to produce antioxidant compounds, were observed by Nabas et al. (2014). Even royal jelly or bee pollen contains glucose and fructose, Vitamin B and its derivates, trace elements such as Fe, Cu, Zn, and Mn. Most them are significant food sources for the probiotic bacterial growth. Haddadin et al. (2012) found that royal jelly supported the growth of *L. acidophilus* and *Bf. animalis spp. lactis* and obtained about 9.0 log cfu/ml probiotic counts when 2 and 5% of royal jelly was added directly to the milk.

That there was an uncertainty concerning the bee pollen addition on the probiotic bacteria (*L. reuteri, L. rhamnosus* and *B. lactis*) was also mentioned by Rosendale et al. (2008). They stated that bee pollen showed a biphasic response, and that the effect of bee pollen on probiotic bacteria was not clear. They also added that the mechanism on bacterial growth by the bee pollen was not yet sufficiently explained. But, Yerlikaya (2014) also found a dose-dependent increase in the growth of probiotic bacteria with the addition of pollen, as was in agreement with my research.

Controversially, no sugar or sugar source as honey was added into the growth media in my research whereas different sugars and honey were used in the experiment by Popa & Ustunol (2011). But, the growth stimulation factors (bee pollen and royal jelly) in my research and the same broth (MRSL broth containing 5 % lactose) as the growth media in both research experiments, were used. As can be seen from the Tables (1-4), the lactic and acetic acid values were close, but slightly lower in the control tubes compared to the findings by Popa & Ustunol (2011). This was probably related to the same broth being used.

Generally, the dose-dependent increase was observed in the probiotic counts due to the bee pollen and royal jelly addition. The probiotic bacterial counts increased less than 1% in most of the samples between 24 and 48 hours after the addition of bee pollen and royal jelly. The bee pollen addition was more effective on the growth of the investigated probiotic bacteria in terms of lactic and acetic acid productions. The incubation time did not make a difference on the acid production of the investigated probiotic bacteria between 24 and 48 hours of incubation. The differences observed in the organic acid productions, mainly D-/L- Lactic acids, were significant between the control and the pollen/ royal jelly added groups ($P \leq 0.05$).

ACKNOWLEDGEMENTS

The author would like to thank Assoc. Prof. Dr. Reyhan Irkin from Balikesir University, Engineering Faculty, Food Engineering Department for her technical assistance and Assist. Prof. Dr. Serdar Duru from Uludag University, Faculty of Agriculture, Department of Animal Science for his support about statistics.

REFERENCES

Bărnuțiu, L. I., Mărghitaș, L. A., Dezmirean, D. S., Mihai, C. M., Bobiș, O. (2011). Chemical composition and antimicrobial activity of royal jelly – Review. *Animal Science and Biotechnologies, 44*(2), 67-72.

Baroutkoub, A., Mehdi, R. Z., Beglarian, R., Hassan, J., Zahra, S., Mohammad, M. S., Mohammad Hadi, E.

(2010). Effects of probiotic yoghurt consumption on the serum cholesterol levels in hypercholestromic cases in Shiraz, Southern Iran. *Scientific Research and Essays, 5*(16), 2206-2209.

Bilisik, A., Cakmak, I., Bicakci, A., Malyer, H. (2008) Seasonal variation of collected pollen loads of honeybees (*Apis mellifera* L. *anatoliaca*). *Grana, 47*(1), 70-77.

Bouzas, J., Kantt, C. A., Bodyfelt, F., Torres, J.A. (1991). Simultaneous determination of sugars and organic acids in Cheddar cheese by high-performance liquid chromatography. *Journal of Food Science, 56*(1), 276–278.

Boylston, T. D., Vinderola, C. G., Ghoddusi, H. B., Reinheimer, J. A. (2004). Incorporation of bifidobacteria into cheeses: challenges and rewards. *International Dairy Journal, 14*(5), 375–387.

Brindza, J., Gróf, J., Bacigálová, K., Ferianc, P., Tóth, D. (2010). Pollen microbial colonization and food safety. *Acta Chimica Slovaca, 3*(1), 95-102.

Dave, R. I. & Shah, N. P. (1998). Ingredient supplementation effects on viability of probiotic bacteria in yogurt. *Journal of Dairy Science, 81*(11), 2804–2816.

FAO/WHO (2006). Probiotics in Food. Health and Nutritional Properties and Guidelines for Evaluation. FAO Food and Nutrition Paper 85. Retrieved April 30- May 1, 2002, from ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf.

FAO (2013a). Royal jelly, value added products from beekeeping, Chapter 6. Retrieved April 5, 2015, from http://www.fao.org/docrep/w0076e/w0076e16.htm.

FAO (2013b). Pollen, value added products from beekeeping, Chapter 3. Retrieved April 5, 2015, from http://www.fao.org/docrep/w0076e/w0076e10.htm.

Goderska, K. & Czarnecki, Z. (2007). Characterization of selected strains from *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. *African Journal of Microbiological Research, 1*(6), 65-78.

Guldas, M. & Irkin, R. (2010). Microflora of yoghurt and acidophilus milk. *Mljekarstvo, 60*(4), 237-243.

Haddadin, M. S. Y., Haddadin, J. & Benguiar, R. (2012). The effect of royal jelly on growth and short-chain fatty acid production of probiotic bacteria and activity of bacterial procarcinogenic enzymes in rat faeces. *Polish Journal of Food and Nutrition Sciences, 62*(4), 251-258. DOI: 10.2478/v10222-012-0058-4

Heller, K. J. (2001). Probiotic bacteria in fermented foods: product characteristics and starter organisms. *American Journal of Clinical Nutrition, 73*(2), 374–379.

Kabeerdoss, J., Devi, R. S., Mary, R. R., Prabhavathi, D., Vidya, R., Mechenro, J., Mahendri, N. V., Pugazhendhi, S., Ramakrishna, B. S. (2011). Effect of yoghurt containing *Bifidobacterium lactis* Bb12® on faecal excretion of secretory immunoglobulin A and human beta-defensin 2 in healthy adult volunteers. *Nutrition Journal, 10*(1), 138-144.

Kacainova, M., Vukovic, N., Chlebo, R., Hascik, P., Katarina, R., Cubon, J., ... Pasternakiewicz, A. (2012). The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia. *Archives of Biological Sciences, 64*(3), 927-934.

Komosinska Vassev, K., Olczyk, P., Kaźmierczak J., Mencner, L., Olczyk, K. (2015). Bee Pollen: Chemical Composition and Therapeutic Application. Evidence-Based Complementary and Alternative Medicine, 2015(1), 1- 6. http://dx.doi.org/10.1155/2015/297425.

Nabas, Z., Haddadin, M. S. Y., Haddadin, J., Nazer, I. K. (2014). Chemical composition of royal jelly and effects of synbiotic with two different locally isolated probiotic strains on antioxidant activities. *Polish Journal of Food and Nutrition Sciences, 64*(3), 171-180.

Olaitan, P. B., Adeleke, O. E. & Ola, I. O. (2007). Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *African Health Sciences, 7*(3), 159–165.

Popa, D. & Ustunol, Z. (2011). Influence of sucrose, high fructose corn syrup and honey from different floral sources on growth and acid production by lactic acid bacteria. *International Journal of Dairy Technology, 64*(2), 247-253.

Rosendale, D. I., Maddox, I. S., Miles, M. C., Rodier, M., Skinner, M., Sutherland, J. (2008). High-throughput microbial bioassays to screen potential New Zealand functional food ingredients intended to manage the growth of probiotic and pathogenic gut bacteria. *International Journal of Food Science and Technology, 43*(1), 2257-2267.

Rudnicka, K., Kwiatkowska, P., Gajewski, A., Chmiela, M. (2015). Honey as a reservoir of *C. botulinum* and a risk factor for infant botulism. *Postepy Mikrobiologii, 54*(2), 184-194.

Shah, N. P. (2000). Probiotic bacteria: selective enumeration and survival in Dairy food. *Journal of Dairy Science*, *83*(4), 894-907.

Shin, H. S. & Ustunol, Z. (2005). Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria: An in vitro comparison. *Food Research International, 38*(6), 721-728.

Shori, A. B. & Baba, A. S. (2015). Survival of *Bifidobacterium bifidum* in cow- and camel-milk yogurts enriched with *Cinnamomum verum* and *Allium sativum*. *Journal of the Association of Arab Universities for Basic and Applied Sciences, 18*(1), 7-11.

Simsek, B., Sagdic, O. & Karahan, A. G. (2002). Importance in Dairy Technology of Bacteriocins Produced by Dairy Starter Cultures. Pamukkale University Engineering College, *Journal of Engineering Sciences, 8*(3), 335-341.

Strus M., Brzychczy-Wloch M., Kochan P., Heczko P. (2004). Hydrogen peroxide produced by *Lactobacillus* species as a regulatory molecule for vaginal microflora. *Medycyna Doświadczalna i Mikrobiologia, 56*(1), 67-77.

Tharmaraj, N. & Shah, N. P. (2003). Selective enumeration of *Lb. delbrueckii ssp. bulgaricus, S. thermophilus, Lb. acidophilus,* Bifidobacteria, *Lb. casei, Lb. rhamnosus* and Propionibacteria. *Journal of Dairy Science, 86*(7), 2288-2296.

Ustunol, Z. & Gandhi, H. (2001). Growth and viability of commercial *Bifidobacterium* spp. in honey-sweetened skim milk. *Journal of Food Protection, 64*(11), 1775-1779.

Vajiheh, F. (2012). Milk Proteins-derived antibacterial peptides as novel functional food ingredients. *Annals of Biological Research,* 3(5), 2520-2526.

Valdez, J. C., Peral, M. C., Rachid, M., Santana, M., Perdigon, G. (2005). Interference of *Lactobacillus plantarum* with *Pseudomonas aeruginosa* in vitro and in infected burns: The potential use of probiotics in wound treatment. *Clinical microbiology and infection, 11*(6), 472-479.

Varga, L. (2006). Effect of acacia (*Robinia pseudoacacia* L.) honey on the characteristic microflora of yoğurt during refrigerated storage. *International Journal of Food Microbiology, 108*(2), 272-275.

Wang, S., Zhu, H., Lu, C., Kang, Z., Luo, Y., Feng, L., Lu, X. (2012). Fermented milk supplemented with probiotics can effectively alter the intestinal microbiota and immunity of host animals. *Journal of Dairy Science, 95*(9), 4813-4822.

Yatsunami K. & Echigo, T. (1985). Antibacterial action of royal jelly. *Bulletin of Faculty of Agriculture, 25*(1), 13-22.

Yerlikaya, O. (2014). Effects of bee pollen supplement on antimicrobial, chemical, rheological, sensorial properties and probiotic viability of fermented milk beverages. *Mljekarstvo, 64*(4), 268-279.