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EFFECTS OF BACTERIOCIN AND ORGANIC ACID ON *Listeria monocytogenes* IN FEED

Ahmet Onder USTUNDAG, Mursel OZDOGAN

Adnan Menderes University, Faculty of Agriculture, Department of Feed and Animal Nutrition, South Campus, Aydın, Turkey, Phone: +90256 772.70.23, Fax: +90256 772.72.33, E-mail: austundag@adu.edu.tr, mozdogan@adu.edu.tr

Corresponding author email: austundag@adu.edu.tr

Abstract

Listeria monocytogenes is a Gram-positive, psychrotropic, facultative anaerobic pathogen and it is the etiological agent of listeriosis, a severe foodborne disease of major public health concern. Listeria monocytogenes has high ability to adapt adverse condition. Therefore, the control of Listeria monocytogenes in foods and feeds is very difficult. N this purpose, organic acids and bacteriocins have been used for many years in preservation of foods against pathogen contamination. In this considering, it was aimed to determine the effects of bacteriocin, organic acid, and mixtures of them on the Listeria monocytogenes in this study. Feeds were divided into six experimental groups that consist of control, 150 mg/kg bacteriocin (B150), 300 mg/kg bacteriocin (B300), 3g/kg organic acid (OA), 150 mg/kg bacteriocin + 3 g/kg organic acid (B150+OA) and 300 mg/kg bacteriocin + 3 g/kg organic acid (B300+OA). Listeria monocytogenes cultures were added by spraying on feed samples in sterile bags and were counted by inoculating onto agars at 0, 7, 15, 21 and 28 days. At the end of this study, bacteriocin and organic acid supplementations have been found to have positive effects on Listeria monocytogenes contamination. But the best antilisterial effects was achieved by combined use of bacteriocin and organic acid.

***Key words**: bacteriocin, organic acid, feed, Listeria monocytogenes.*

INTRODUCTION

Animal feeds play a key role in establishing the infrastructure necessary for the production of animal origin products in the global food industry and any factor that affects the security of feed can directly affect the animal health and human health through the food chain. Therefore, safe feed production is an essential element to reduce and prevent food safety hazard into the food chain (FAO and IFIF, 2010; Bryden, 2012). However, feeds can be contaminated with pathogenic bacteria in some cases. The main sources of contamination are grains and animal origin ingredients (Maciorowski et al., 2006; Sapkota et al., 2007; Cegielska- Radziejewska et al., 2013). These ingredients can become cantaminated at any time during growing, harvesting, processing, storage and distribution (Maciorowski et al., 2007; Carrique-Mas et al., 2007; Davies and Wales, 2010; Jones, 2011; Torres et al., 2011; Berge and Wierup; 2012). *Salmonella* spp. was identified as the major hazard for microbial contamination of animal feed by the Panel on Biological Hazards. *Listeria monocytogenes*, *Escherichia coli* O157: H7 and *Clostridium* sp. are other hazards for which feed is regarded as a far less important source (EFSA, 2008).

Listeria monocytogenes is an important ubiquitous Gram-positive pathogen that causes listeriosis, has a high ability to adapt to adverse conditions and can survive at temperatures of 0-45 °C (Bizani et al., 2008; Dabour et al., 2009; Cosentino et al., 2012; Kasra-Kermanshahi and Mobarak-Qamsari, 2015; Osés et al., 2015; Camargo et al., 2016). These characteristics of *Listeria monocytogenes* make its control extremely difficult (Iseppi et al., 2008). Listeriosis is known as a serious disease which has a high mortality rate as high as 30% for especially pregnant women, newborn children and the elderly and individuals with a weakened immune system (Halimi et al., 2010; Kosonosoka et al., 2012; Kim et al., 2015; Pilchová et al., 2016). Consequently, the control of pathogenic microorganisms in food and feed has become a major concern in the whole world (Halimi et al., 2010).

Organic acids have been used for many years in preservation of food and feed (Carrique-Mas et al., 2007). In addition, bacteriocins, which are ribosomally synthesized proteinaceous compound, that have a bactericidal or bacteriostatic effect on similar or closely related bacteria strains, have begun to be used as alternative to antibiotics in animal husbandry (Lim and Kim, 2009; Sirsat et al., 2009; Kos et al., 2011; Toomula et al., 2011; Arokiyamary and Sivakumar, 2012; Murtaza et al., 2012; Musikasang et al., 2012; Yang et al., 2012; Chowdhury et al., 2013; Schyns et al., 2013; Yusuf and Hamid, 2013).

All these considering, the purpose of this study was to determine antilisterial effects of bacteriocin and organic acid on *Listeria monocytogenes* in feeds.

MATERIALS AND METHODS

Feeds were divided into six experimental groups that consist of control, 150 mg/kg bacteriocin (B150), 300 mg/kg bacteriocin (B300), 3 g/kg organic acid (Selacid® Green Growth MP) (OA), 150 mg/kg bacterocin + 3 g/kg organic acid (B150+OA), 300 mg/kg bacteriocin + 3 g/kg organic acid (B300+OA). Active ingredients of Selacid ® Green Growth MP were sorbic acid, formic acid, acetic acid, lactic acid, propionic acid, ammonium formate, citric acid, 1,2-propanediol, coconut/palm kernel fatty acid distillate, silicondioxide (SiO_2). Nisin was used as bacteriocin in this study.

Culture of *Listeria monocytogenes* was grown in tryptic soy broth at 37°C for 24 h and were diluted $1:10^{-3}$ in sterile saline solution to a final concentration of approximately 1.0 log cfu/ml and 50 ml of this solution added by spraying whilst stirring the 150 g of feed samples which were weighed into sterile bags. Feed samples were kept at room temperatures during the experiment. On 0^{th}, 7^{th}, 15^{th}, 21^{th} and 28^{th} days of the study, 25 g of feed samples were taken and homoginated with 225 ml of Listeria Enrichment Broth and incubated at 30°C for 48 h. Then, samples were serially diluted in sterile saline solution and 10 µl of these solutions were pipetted onto PALCAM Listeria Selective Agar with supplement (Merck) in triplicate and plates were incubated at 37°C for 48 h. Colonies of *Listeria monocytogenes* were enumerated and results expressed as CFU/g.

The values did not adhere to normal distrubition, so data was expressed in log 10. Data were analysed by ANOVA using the GLM procedure with SAS 8 software. The differences among the means were tested using Duncan's multiple range tests. All statements of significance were based on a probability of $P<0.05$.

RESULTS AND DISCUSSIONS

Effects of bacteriocin and organic acids on *Listeria monocytogenes* growth in feed were given in Figure 1.

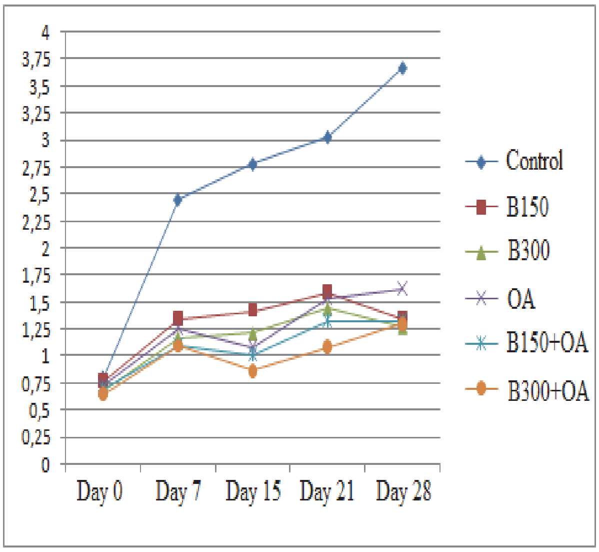

Figure 1. Changes in the number of *Listeria monocytogenes* in feeds (log 10/g).

There were no significant differences between the groups in the initial numbers of *Listeria monocytogenes* ($p>0.05$). The number of *Listeria monocytogenes* in control feed was found to be higher than other groups on the 7^{th} day of the trial ($p<0.05$). There was no statistical differences between experimental groups. On the 15^{th} day, experimental groups decreased *Listeria monocytogenes* count compared to control group ($p<0.05$). It was found that bacteriocin and organic acid supplemented groups had greatest antilisterial activity in this period ($p<0.05$). Similar results was observed on 21^{th} and 28^{th} day of the trial.

In this study, it was observed that the increase in bacteriocin levels decreased *Listeria monocytogenes* count on 7^{th}, 15^{th}, 21^{th} and 28^{th} days of trial compared to control group.

However, 300 mg/kg bacteriocin supplemented group (B300) was statistically more effective than 150 mg/kg bacteriocin supplemented group (B150) only on 15^{th} day of trial ($p<0.05$). There was no significant difference between bacteriocin supplemented groups on other days of trial. The studies conducted to investigate the antibacterial effects of bacteriocin on feed and feed ingredients are deficient. However, certain studies reported that addition of bacteriocin producing microorganism to silage leads to decrease in *Listeria monocytogenes* count (Marciňáková et al., 2008; Zielińska et al., 2015). Also, the results of various trials indicated that addition of bacteriocin or bacteriocin producing microorganism decreased *Listeria monocytogenes* count in milk (Bizani et al., 2008; Chanos and Williams, 2011; Cosentino et al., 2012; Fatima and Mebrouk, 2013; Han et al., 2013; García et al., 2015), cheese (Mahmoudi et al., 2012; Malheiros et al., 2012; Yang et al., 2012; Fernández, et al., 2014; Hassanzadazar et al., 2014), yogurt (Benkerroum et al., 2003; Aslım et al., 2004; Tufail et al., 2011; Zaeim et al., 2014) and meat (Enan, 2006; Dortu et al., 2008; Ruiz et al., 2009; Lauková and Turek, 2011; Sant'Anna et al., 2013; Osés et al., 2015; Zanette et al., 2015). The results of this trial are in agreement with the findings reported by previous researchers. It was indicated that the antibacterial effect of bacteriocin is based on different mechanisms of action such as alteration of enzymatic activity, inhibition of spore germination and forming membrane pores that disturb the energy potential of sensitive cells (Parada et al., 2007; Singh et al., 2013). But these mechanisms can be influenced by several factors like bacteriocin dose and degree of purification, physiological state of the indicator cells and experimental conditions (Cintas et al., 2001).

It was observed that the number of *Listeria monocytoges* in feeds containing organic acid was significantly lower than the control group in this trial. These results are in agreement with previous studiexaminnig the effects of organic acids on the *Salmonella*, *Clostridium*, *Enterobacter* in feed (Carrique-Mas et al., 2007; Casagrande et al., 2013; Koyuncu et al., 2013; Sbardella et al., 2015) and on *Listeria monocytogenes* in foods as protective (Glass et al., 2013; Sansawat et al., 2013; Morey et al., 2014). Ricke (2003) reported that antibacterial effects of organic acids may be due to various mechanism such as reducing intracellular pH and denaturation of acid-sensitive proteins and DNA.

In this trial, the best antibacterial effects against to *Listeria monocytogenes* was observed in groups that bacteriocin and organic acid were added together. There are no available references about feed to support these findings. But, there are various studies investigating the effects of bacteriocin and organic acid supplementation together on pathogenic bacteria in foods. The results of these studies reported that bacteriocin and organic acid supplementation have beneficial effects in preventing pathogenic microorganisms growth (Bari et al., 2005; Molinos et al., 2005; Wan Norhana et al., 2012).

The increase in antibacterial activity by the addition of a mixture of bacteriocin and organic acid to the feeds indicates that these two additives have a synergistic effect on *Listeria monocytogenes*.

CONCLUSIONS

Although the positive effects were observed with use of bacteriocin and organic acid supplementation, the best antilisterial effect was achieved by combined use of bacteriocin and organic acid in our study. In order to better understand the effect mechanisms of these additives in animals and feeds, additional researches on different concentrations, different environments conditions with different species of bacteria are needed.

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