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Full Length Research Paper

Effect of probiotics on microbial level in Azerbaijan native duck (*Anas platyrhynchos*) meat

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Probiotics are products of microbial cells that have useful effect on health and tranquility of human. According to several studies, valuable properties such as anti-carcinogenic, anti-mutagenic, increasing body immunity and resistance against entero-pathogens have been related to probiotics. Hence, the aim of this study was to specify the effect of probiotics usage in duck diet on microbial hazards of meat. In order to achieve this aim, two groups of 40 ducks each were used as treatment and control groups kept in the same condition. For the experiment, a 42-day period were chosen. Edible probiotic was given to treatment groups, then both groups were slaughtered in the slaughter house and about 100 g of skin and meat samples were taken from each carcass aseptically and then taken to food Hygiene laboratory of Veterinary Faculty and using the standard methods of 8923-1, 1194, 2198, 2197, 437, 1810, 2946, the total microbial count, *Staphylococcus aureus* count, fecal Streptococci count, *Clostridium perfringens* count, coliform count and detection of Salmonella and *Escherichia coli* were studied in the samples. The results were analyzed with the statistic test of Independent T-test and Chi-square test. The comparison of the means of microbial count in the control and treatment groups showed a significant reduction (P<0.005). Furthermore, detection of *E. coli* showed a significant reduction in the treatment group, but reductions of Salmonella and Clostridium had no significant difference. Consequently, it seems that using oral probiotics in diet causes reduction in bacterial entero-pathogens of duck meat. Therefore, if the frequency and contamination level of these microorganisms could be reduced in duck meat, it will be an effective step in the reduction of food born disease, meat hygienic quality and shelf life of duck meat.

Key words: Probiotic, hazards, duck, meat.

INTRODUCTION

The word “Probiotic” is a Greek word that means life, and according to definition, probiotics are named as products or components of the microbial cells that have useful effect on health and tranquility of human (Mirzaei, 2004; Bendixen, 2005). Probiotics, as a live microbial food supplement, have beneficial effects on health of their host by improving the microbial balance in the digestive tract (Bisson et al., 2010). Supplementation of probiotic preparative on of broiler ducks has been reported (Weis et al., 2008). The useful effects of probiotics have been shown to include the prevention of pathogens such as Salmonella (Ogawa et al., 2001) and in prevention of traveler’s diarrhea (Hilton et al., 1997). The inhibitory effect of *Lactobacillus casei* on the connection and adhesion of *Escherichia coli* O_{101}: K_{99} to the intestinal mucosa of lambs (devoid of microbes) has been tested (Bomba et al., 1997). Also, researches have indicated that *L. casei* can be colonized probably as a probiotic in the gastrointestinal tract of murine model and reduced colonization of enterotoxigenic *E. coli* (ETEC) (Mirnejad et al., 2010).

Some of the proven potential effects of probiotics include aiding digestion of lactose in the intestine, inhibitory effects on colon cancer, strengthen the immune system, stimulation of the small intestine growth, prevention of hypersensitive reactions and resisting the growth pathogens in the intestine, among others (Montrose et al., 2005; Ouwehand et al., 2002; Burns and

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Table 1. Mean standard deviation and standard error of total microbial count logarithm of duck meat in control and treatment groups.

Parameter	Group	Number	Mean log cfu/g	Standard deviation	Standard error
Total microbial count	Treatment	40	3.451	0.60673	0.10731
	Control	40	4.4785	0.75032	0.13001
Staphylococcus aureus count	Treatment	40	3.554	1.01245	0.16585
	Control	40	4.0355	0.5285	0.10506
Fecal streptococci count	Treatment	40	2.1412	0.25653	0.03243
	Control	40	3.8063	0.36212	0.04882
Coliform count	Treatment	40	1.5744	0.47501	0.10153
	Control	40	4.1012	0.78271	0.15051
Clostridium perfringens count	Treatment	14	1.1426	0.11513	0.07097
	Control	13	1.007	0.0316	0.0398

Rowland, 2000). The extensive usage of the probiotics as food supplement in human and animal food, especially sporogenic bacteria such as Bacillus, has been suggested in poultry industry and fisheries. Also, their use in the prevention of intestinal gastroenteritis and decreasing of the entero-pathogens activities has been recommended. In addition to competition in growth, Bacillus does this performance with production of antimicrobial substances in the intestine (Hong et al., 2005). Furthermore, the European Union has emphasized the use of probiotic as food additives for sanitary and safety purposes in poultry. Researches showed that probiotics diminish the mortality rate, increase the weight and laying in poultry and increase the growth and fertility of turkey and growth and milk production in ruminants (Anadon et al., 2006). Besides, scientist have observed that consumption of probiotics up to 10 times of the normal values does not have negative effect on the consumer (Anadón et al., 2006).

Enterococcus faecium isolated from chickens crop, has the ability to produce bacteriocin and act against poultry disease like *Salmonella pullrum*, *Enterococcus hirae* and *Listeria monocytogenes*. Also, the S-layer proteins of *Lactobacillus cryspatus* are responsible for competing with *E. coli* O_{157}:H_7 and *Salmonella typhimurium* (Gordon, 2002). Hence, the purpose of this study was to examine the effect of probiotic usage in duck diet on microbial levels in the duck meat.

MATERIALS AND METHODS

Studied populations were duck of one month old divided into two groups and selected randomly as the treatment and control groups (each group had 40 duck). The experiment was conducted for a period of 42 days. Oral probiotic (protexin) was fed to the treatment group and this probiotic contained 2×10^9 microorganisms/g, included 9 strains (*S. feacium, Streptococcus thermophilus, Lactobacillus plantarum, Lactobacillus johnsonii*, *Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium bifidum, Aspergillus ourozai* and *Candida pentolopsy*). Protexin consumption began from the first day of ducks breeding, as in first 7 days, 1 g/L of water and after one week (beginning of starter period) 150 g per each tone of feed, 100 g per each ton of feed in growth period, for 50 g of protexin per each tone of feed, in final period manually was mixed and was eaten to ducks.

After the end of the breeding period, the ducks were slaughtered in the slaughterhouse, and we sampled from each duck about 100 g of skin and meat from the breast area which were transferred to the food laboratory of Veterinary Medicine Faculty of Islamic Azad University of Tabriz for microbial culture. According to standard methods (numbers 8923-1, 1194, 2198, 2197, 437, 1810 and 2946), total microbial count, *Staphylococcus aureus* count, fecal streptococci count, detection of Salmonella and *Clostridium perfringens* were performed in the laboratory (ISIRI nos. 8923-1; 437; 2946; 2198; 1194; 2197 and 1810). For comparing the quantitative data of two groups, independent T-test and for comparing of the qualitative data, Chi-square test was used.

RESULTS AND DISCUSSION

Logarithm of the mean total microbial count, *S. aureus*, and fecal streptococci count, Salmonella, coliform and *C. perfringens* of meat were calculated in the treatment and control groups separately, with the standard deviation and standard error was specified in each case (Table 1). Considering that *C. perfringens* was not isolated in some samples, the existence or inexistence of this bacterium in samples with Salmonella and *E. coli* was calculated in treatment and control groups (Table 2).

Comparison of the means of total microbial count, coliform count, fecal Streptococci and *S. aureus* count in meat showed a significant decrease by independent statistical T-test in the treatment and control groups ($P < 0.005$) but this comparison did not show any significant decrease for *C. perfringens* ($P > 0.05$). Also, existence

Table 2. Relative frequency of Salmonella, *E. coli* and *Clostridium perfringens* of duck meat in treatment and control groups.

Group		Number	Treatment	Control
Salmonella	+	40	3	6
	-	40	37	34
E. coli	+	40	23	40
	-	40	17	0
Clostridium perfringens	+	40	14	13
	-	40	26	27

frequency of *E. coli* showed a significant reduction in the case of the control group ($P < 0.05$), while this reduction in Salmonella and Clostridium was not significant ($P > 0.05$). Therefore, probiotic usage in the diet of breeding duck reduces the microbial population of some pathogens significantly. Primary microbial contamination rate of raw meat is variable in the amplitude of 10^2 - 10^4 cfu/g that 10% of the microbial population may be able to grow in lower temperatures and the less percentage of them to meat spoiling. In fresh or raw meat, air, soil, skin, hair, wool and animal gastrointestinal tract are the main pollutant sources of carcasses in or after the slaughtering process. In the exposition locations of meat, in addition to the above factors, workers contact with meat, dishes and cutting machines are considered as the most important factors of meat pollutants (Dickson and Anderson, 1992).

It has been reported that the oral consumption of probiotic with the value of 0, 0.1 and 0.2% of *S. cerevisiae* in poultry meat for 49 days, and afterward slaughtered and meats kept in 3°C temperature for 12 days in two-mode, vacuum and normal, decreases sacrophilic bacteria and Enterobacteriaceae, but it increases the total microbial count (Aksu et al., 2005). These results were according to the result of our findings, except for the total count. Researchers have shown that inflammatory bowel diseases like irritable bowel syndrome may be created or intensified by the change of intestinal flora (which includes putrefaction) (Shanahan, 2000). Intestinal microflora plays a vital role in inflammation of intestine and probiotics can cure this condition through the micro flora reformation (Bomba et al., 1997). Lactobacilli consumption has increased the IgM and IgA levels in the host body. Also, according to a previous report, Bifidobacterium species stimulate the synthesis of IgA in laboratory conditions. Consumption of *L. Johnsonii* through the fermentative milk strengthens phagocytosis. More also, in a prospective study, scientists have proven that the consumption of fermentative milk strengthen the immunity system against virus antigen.

These studies confirm the hypothesis that *L. casei* stimulates special immune response (Gordon, 2002). Therefore, according to the findings of our study, probiotics and especially Lactobacilli eliminate or reduce the entero-pathogenic microbes. Also, the preparation of the probiotic Propoul with strain *Lactobacillus fermentum* manifested as a preparative with higher effect on the meat production in comparison with the probiotic Protexin concentrate with strain of *Enterococcus faecium* (Weis et al., 2008). In a laboratory research, it was obvious that *L. casei shirota* in competing with gastrointestinal bacteria prevented the connection of them to the surface of Caco-2 cells about 46%. This research identified that the most inhibition of LCS (above 30%) is on *E. coli, S. typhimurium* (Matsuzaki et al., 1998). Recently, many studies have reported the antagonistic effects of lactic acid bacteria (such as, *Lactobacillus* sp., *Streptococcus* sp. and *Bifidobacterium* sp.) and other microbial agents including *Clostridium* sp., *Enterococcus* sp. and yeast against various pathogenic bacteria (Hickson et al., 2007). Hence, probiotics can be regarded as green feed additives in meat duck diet to replace antibiotic (Jing and You, 2004).

Since the major part of the microflora of meat are intestinal microorganisms, the aforementioned articles have so far confirmed the inhibitory effect of probiotic on growth and proliferation of Intestinal microorganisms by stimulation of immunity system mechanisms, production of bacteriocin, microbial competition, etc. Our results therefore confirm these studies about the reduction of microbial count of meat in the treatment group by oral consumption of probiotic. Despite the inhibition of Salmonella and Clostridium by probiotic bacteria, significant changes were not observed in this study, which may probably be due to the low prevalence of these germs in the meat of the studied duck and size of samples.

Conclusion

From the findings of this study, it is suggested that the use of probiotics in duck breeding industry could reduce the microbial hazards in meat. In spite of restrictions on their usage including the effect of probiotics on the host and digestive microflora, transferring of harmful genes, the shape of products, the value and consumption

method of probiotics and age and species of animal etc., and considering the European Union emphasis on the application of probiotics, it is proposed and recommended that probiotics be used in Iran.

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