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Full Length Research Paper

Effects of probiotic (live and inactive *Saccharomyces cerevisiae*) on meat and intestinal microbial properties of Japanese quails

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The present work evaluated the effect of probiotic (live and inactive *Saccharomyces cerevisiae)* on meat and intestinal microbial properties of Japanese quails. Twenty-four (24) 1-day-old Japanese quails were obtained from a commercial hatchery. The birds were randomly divided into 2 groups. The dietary treatments were: 1) basal diet (control), 2) basal diet plus 0.1% live *S. cerevisiae* and 0.05% inactive *S. cerevisiae.* The Japanese quails were fed with the diets from day 1 to day 72. At the end of the experiment, 12 Japanese quails per experimental group were slaughtered, and meat and intestinal samples were taken. Collected meat and intestinal samples were transported at 4°C to the laboratory of food hygiene in Islamic Azad University, Tabriz branch. In this study, each sample of 25 g was prepared according to the standard methods of Institute of Standards and Industrial Research of Iran; No: 356, 1810, 2197, 2946, 1194 and 437 for preparation, culture and detection of bacterial total count, *Lactobacilli* bacteria, *Coliforms* bacteria, *Clostridium perfringens, Staphylococcus aureus* and *Streptococcus* sp. According to the results of effects of probiotic (active and inactive *S. cerevisiae*) on intestinal and meat microbial properties of Japanese quails, in the probiotic cases, a significant reduction in the properties of total bacterial count (p = 0.007), *Streptococcus* sp. (p = 0.046), *Coliform* (p = 0.041) and *Lactobacillus* (p = 0.032) in intestinal microbial properties and only significant reduction on properties of total bacterial count was observed (p = 0.01). Probiotics may help in reducing the microbial properties of meat and intestine, and the present study had provided evidences that supplementation of probiotics in the diet of Japanese quails had a significant effect on microbial properties reduction, especially on intestinal microbial flora.

Key words: Probiotic, *Saccharomyces cerevisiae,* microbial properties, Japanese quails.

INTRODUCTION

Recent outbreaks of food-borne diseases show the need to reduce bacterial pathogens in foods of animal origin. Animal enteric pathogens are a direct source for food contamination (Gaggia et al., 2010). The ban of antibiotics as growth promoters (AGPs) has been a challenge for animal nutrition which increases the need to find alternative methods to control and prevent pathogenic bacterial colonization. There is currently a world trend to reduce the use of antibiotics in animal food due to the contamination of meat products with antibiotic residues, as well as the concern that some therapeutic treatments for human diseases might be jeopardized due to the appearance of resistant bacteria (Mohan et al., 1996). The chicken industry is one of the most dynamic of the world agribusiness trade. The importance of feed supplementation in poultry production has increased in the last years with the aim of improving the economic

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situation of poultry projects. Nowadays, food safety is more seriously considered than before. On the other hand, antibiotics and hormones have been used in feeds for stimulation of animal performance. However, these promoters have undesirable side effects such as toxicity, allergy, cancer, drug resistance and residues in food (Ibrahim et al., 2005). Their use in practice is therefore either diminished or banned. The use of natural growth promoters has increased in many countries over the last 15 to 20 years. However, the research on feeding partridges, especially with relation to feed additives, is limited. In the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and diseases so as to improve meat and egg production. However, the use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1999) and imbalance of normal microflora (Andremont, 2000). As a consequence, it has become necessary to develop alternatives using either beneficial microorgansims or nondigestible ingredients that enhance microbial growth. In broiler nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida* and *Saccharomyces* have a beneficial effect on broiler performance (Ashayerizadeh et al., 2009; Kabir et al., 2004; Pelicano et al., 2003), modulation of intestinal microflora, pathogen inhibition, intestinal histological changes, immunomodulation, certain haematobiochemical parameters, improving sensory characteristics of dressed broiler meat and promoting microbiological meat quality of broilers (Islam et al., 2004; Khaksefidi and Ghoorchi, 2006; Mountzouris et al., 2007; Higgins et al., 2007; Chichlowski et al., 2007; Haghighi et al., 2005). Recently, alternatives for substituting these traditional growth promoters have been evaluated and probiotics have been the most studied. Animals can be seriously impacted by bacterial pathogens that affect growth efficiency and overall health, as well as food safety. Several of these pathogens, such as Salmonella, can be a shared problem for both human and animal health, and have been isolated from multiple animal species. The intestinal microbial population of animals is very dense and highly diverse (Kabir, 2009). Probiotic is a relatively new word meaning 'for life', which is used to name microorganisms that have beneficial effects on humans and animals. These microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus, Pediococcus* and some yeasts have also been found as suitable candidates. Together, they play an important role in the protection of the organism against harmful microrganisms and also strengthen the host's immune system. Probiotics therapy (for some illnesses), which destroys can be found in dairy and non dairy products. They are usually consumed after the antibiotic the microbial flora present in the digestive tract (both the useful and the targeted harmful microbes). Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the population of useful or beneficial microbes in the intestinal flora (Ricardo S et al., 2010). The probiotic potential of different bacterial strains, even within the same species, differs. Different strains of the same species are always unique, and may have different areas of adherence (site-specific), specific immunological effects, and actions on a healthy vs. an inflamed mucosal milieu which may be distinct from each other. Current probiotic research aims at the characterization of the normal, healthy gut microbiota in each individual, assessing the species composition as well as the concentrations of different bacteria in each part of the intestine. The target is to learn how to understand host-microbe interactions within the gut, microbe-microbe interactions within the microbiota and the combined health effects of these interactions. The goal is to define and characterize the microbiota both as a tool for nutritional management of specific gut-related diseases and as a source of new microbes for future probiotic bacteriotherapy applications. This may eventually include organisms specifically isolated to provide site-specific actions in disorders such as the irritable bowel syndrome. Nowadays, consumers are aware of the link among lifestyle, diet and good health, which explains the emerging demand for products that are able to enhance health beyond providing basic nutrition. The list of health benefits accredited to functional food continues to increase and the probiotics are one of the fastest growing categories within food for which scientific studies have demonstrated therapeutic evidence. Among several therapeutic applications of the probiotics that can be cited are the prevention of urogenital diseases, alleviation of constipation, protection against traveller's diarrhoea, reduction of hypercholesterolaemia, protection against colon and bladder cancer, prevention of osteoporosis and food allergy. One of the most studied strains, *Bifidobacterium lactis,* has been used in several types of studies to demonstrate its probiotic ability, and scientific evidence for this strain has been cited in many reviews.

Recent studies have suggested that probiotics have demonstrated beneficial effects on human and animal health. Most of the clinical probiotic research has been aimed at infantile, antibiotic-related and traveller's diarrhoea. The non-pathogenic organisms used as probiotics consist of a wide variety of species and subspecies, and the ability to adhere, colonize and modulate the human gastrointestinal system is not a universal challenge. *Lactobacillus* and *Bifidobacterium* are the main probiotic groups; however, there are reports on the probiotic potential of yeasts. Some of the identified probiotic strains exhibit anti-inflammatory, anti-allergic and

Table 1. Ingredients of basal diet.

Ingredients	Percentage
Yellow corn	53.00
Soybean meal, 44% CP	37.00
Fish meal, 60% CP	5.50
Vegetable oil	1.00
Oyster shell	1.00
Mono calcium phosphate	1.50
DL-Methionine	0.15
Sodium chloride	0.15
Mineral-vitamin premix	0.50
Vitamin A	0.10
Vitamin E	0.10

other important properties. Besides, the consumption of dairy and nondairy products stimulates the immunity in different ways. The use of probiotics for meat and carcass quality improvement has been questioned and many unclear results have been shown. Some authors reported advantages of probiotic administration, whereas others did not observe improvement when probiotics were used (Saadia and Hassanein, 2010). Hence, the aim of this study was to evaluate the effects of probiotic (live and inactive *Saccharomyces cerevisiae)* on meat and intestinal microbial properties of Japanese quails.

MATERIALS AND METHODS

Birds and housing

Twenty four 1-day-old Japanese quails were obtained from a commercial hatchery. The birds were randomly divided into 2 groups (12 birds/group) and housed in pens of identical size (1.75 × 6 m) in a deep litter system with a wood shaving floor. Each bird had free access to water and feed. The climatic conditions and lighting program were computer-operated and followed the commercial recommendations. Environmental temperature in the first week of life was 35°C and decreased to 25°C until the end of the experiment (unit 72 days).

Dietary treatments

The dietary treatments were: 1) basal diet (control), 2) basal diet plus 0.1% live *S. cerevisiae* and 0.05% inactive *S. cerevisiae.* The Japanese quails were fed with the diets from day 1 to day 72. Basal diet of the control group is shown in Table 1.

Meat and intestinal samples preparation

At the end of the experiment, 12 Japanese quails per experimental group were slaughtered, meat and intestinal samples were taken, then microbiological experimentation procedure was done as follows: 50 g of meat and intestinal samples were adjustely weighed and transferred into test tube containing 9 ml of 0.1 sterile peptone, the samples were mixed well and serial dilutions were prepared.

Enumeration of bacteria

Collected meat and intestinal samples were transported at 4°C to the laboratory of food hygiene in Islamic Azad University, Tabriz branch. In this study, each sample of 25 g was prepared according to the standard methods of Institute of Standards and Industrial Research of Iran; No: 356, 1810, 2197, 2946, 1194 and 437 for preparation, culture and detection of *Bacterial* total count, *Lactobacilli* bacteria, *Coliforms* bacteria, *Clostridium perfringens*, *Staphylococcus aureus* and *Streptococcus* sp.

Statistical analysis

Statistical analyses were conducted with the variance, Chi-square tests and SPSS program (version 12).

RESULTS AND DISCUSSION

Results of this study are divided into 2 parts (intestine and meat):

Intestinal microbial properties of Japanese quails

According to the results of the effects of probiotic (live and inactive *S. cerevisiae)* on intestinal microbial properties of Japanese quails, in the probiotic cases, a significant reduction on properties of total bacterial count ($p = 0.007$), *Streptococcus* sp. ($p = 0.046$), *Coliform* ($p = 0.041$) and *Lactobacillus* ($p = 0.032$) was observed, but a significant effect on *S. aureus* and *C. perfringens* properties was not observed (Table 2).

Meat microbial properties of Japanese quails

With regards to the results of the effects of probiotic (live and inactive *S. cerevisiae)* on meat microbial properties of Japanese quails, only significant reduction on properties of total bacterial count was observed ($p = 0.01$) and significant effect on reduction of the properties of *Streptococcus* sp., *Coliform*, *Lactobacillus*, *S. aureus* and *C. perfringens* was not observed (Table 3).

Probiotic strains have been shown to inhibit pathogenic bacteria both *in-vitro* and *vivo* through different mechanisms. The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora by competitive exclusion and antagonism, (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production, (iii) improving feed intake and digestion, (iv) stimulating the immune system (Kabir, 2009). Kabir et al. (2005) attempted to evaluate the effect of probiotics with regard to clearing bacterial infections and regulating intestinal flora by determining the total viable count (TVC) and total *Lactobacillus* count (TLC) of the crop and cecum samples of probiotics and conventional fed groups at the

Table 2. Effects of probiotic (live and inactive *S. cerevisiae*) on intestinal microbial properties of Japanese quails.

Bacterial	*Control			Probiotic			Significance
	N	Mean	SD	N	Mean	SD	
Total bacterial count	12	8.2109	1.16991	12	5.6959	0.39534	0.007
Staphylococcus aureus	12	2.2470	1.78261	12	0.7964	1.36970	0.402
Fecal *Streptococcus*	12	2.9075	2.6669	12	0.8731	1.3496	0.046
Clostridium perfringens	12	4.7026	1.48716	12	3.3873	1.6398	0.292
Fecal *Coliforms*	12	6.1510	3.01262	12	2.3229	2.5282	0.041
Lactobacillus	12	2.8629	2.3738	12	4.6674	2.5808	0.032

*, Group.

Table 3. Effects of probiotic (live and inactive *S. cerevisiae*) on meat microbial properties of Japanese quails.

Bacterial	*Control			Probiotic			Significance
	N	Mean	SD	N	Mean	SD	
Total bacterial count	12	3.8594	0.4289	12	2.7968	0.6555	0.01
Staphylococcus aureus	12	2.0261	0.5284	12	1.6667	0.4211	0.835
Fecal *Streptococcus*	12	5.2761	1.1648	12	4.9114	1.1298	0.947
Clostridium perfringens	12	1.9262	1.7535	12	1.7052	0.9984	1.00
Fecal *Coliforms*	12	3.1056	0.8991	12	2.2473	0.3921	0.21
Lactobacillus	12	1.4365	0.9186	12	1.6202	1.0824	1.00

***,** Group.

2nd, 4th and 6th week of age. Their result revealed competitive antagonism. The result of their study also showed that probiotic organisms inhibited some non-beneficial pathogens by occupying intestinal wall space. They also demonstrated that broilers fed with probiotics had a tendency to display pronounced intestinal histological changes such as active movement in cell mitosis and increased nuclear size of cells, than the controls (Kabir et al., 2005). It is well recognized by this time that the probiotics are live microorganisms and when administered through the digestive tract, cause a positive impact on the host's health. Studies on the beneficial impact on poultry performance have indicated that probiotic supplementation can have positive effects. Kabir et al. (2004), for example, conducted a 6 weeks growth performance study with broilers and found that live weight gain and carcass yields were significantly higher in broilers fed probiotic supplementation (Kabir et al., 2004).

A study was done by Sadiaan et al. (2010) on the effect of adding probiotic (*S. cerevisiae*) to diets on intestinal microflora and performance of hy-line layers hens, and the results showed that adding live yeast at 0.4 or 0.8% into laying hen diets can enhance the productive performance and nutrients utilization via the inhibitory effect of yeast against pathogenic bacteria and also reveals that probiotics could be successfully used as nutritional tools in poultry feeds for the promotion of growth, modulation of intestinal microflora, pathogen inhibition, immunomodulation and promotion of meat quality of poultry. The antagonistic effect of live yeast against intestinal microflora was elucidated by Line et al. (1998) that several harmful pathogenic bacteria have been shown to exhibit a specific binding for the sugar mannose. A live yeast cells contain mannose in their wall. This mannose in the cell wall may cause the yeast to act as a decoy for the attachment of pathogens. Because yeast has been demonstrated not to permanently colonize animals, the yeast and any yeast-bound pathogens are passed out in the bird through excretion and bacterial colonization is diminished (Line et al., 1998).

Kabir et al. (2004) reported that probiotic microorganisms, once established in the gut, may produce substances with bactericidal or bacteriostatic properties (bacteriocins) such as lactoferrin, lysozyme, hydrogen peroxide as well as several organic acids. These substances have a detrimental impact on harmful bacteria, which is primarily due to lowering of the gut pH. A decrease in pH may partially offset the low secretion of hydrochloric acid in the stomach. In addition, competition for energy and nutrients between probiotic and other bacteria may result in suppression of pathogenic species Numerous factors such as animal to animal variation, strain of yeast and experimental procedures have contributed to the variation in the results of yeast culture studies. However, the digestive advantages of enhanced nutrient digestibility, cecal fermentation and subsequent production parameters provide justification for nutritionists to continue to research on yeast culture supplementation (Kabir et al., 2004).

In a study by Pelicano et al. (2003) on the effect of different probiotics on broiler carcass and meat quality, one thousand and fifty male Cobb chicks were distributed at one day of age in a randomized design with 3 * 2 + 1 factorial arrangement (3 probiotics, 2 levels of probiotics in drinking water and 1 negative control group), using 5 replications with 30 birds. Carcass yield was higher ($p<0.05$) in control birds. Nevertheless, the groups fed with probiotics showed higher ($p<0.01$) leg yield at 45 days of age. There was a significant decrease in color (lightness) and increase in pH of the breast muscle 5 h after slaughter in the probiotics treated birds. In the sensory analysis, meat flavor and general aspect 72 h after slaughter were better when probiotics were added in both water and diet. There were no differences in water holding capacity, cooking loss and shearing force among different probiotics or between them and the control. Thus, meat quality was better when probiotics were added to the water and diet instead of only the diet. Nevertheless, carcass and meat quality showed no alteration when the control group was compared to the birds fed with probiotics, except for leg yield improvement in the latter (Pelicano et al., 2003).

Conclusion

Probiotics may help in reduction of the microbial properties of meat and intestine, and the present study has provided evidences that supplementation of probiotics in diet of Japanese quails have a significant effect on microbial properties reduction, especially on intestinal microbial flora.

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