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Full Length Research Paper

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

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The present work evaluated the effect of probiotics (live and inactive *Saccharomyces cerevisiae*) on the meat and intestinal microbial properties of Japonica quail. Twenty-four one-day old Japonica quail were obtained from a commercial hatchery. The birds were randomly divided into 2 groups. The dietary treatments given to the birds included basal diet (control) and basal diet plus 0.1% live *S. cerevisiae* and 0.05% inactive *S. cerevisiae*. The Japonica quail were fed with the diets from days 1 to 72. At the end of the experiment, 12 Japonica quail per experimental group were slaughtered, and meat and intestinal samples were taken. The collected meat and intestinal samples, kept at 4°C, were transported to the laboratory of food hygiene in Islamic Azad University, Tabriz Branch. In this study, the detection of bacterial total count, *Lactobacilli, Coliforms, Clostridium perfringens, Staphylococcus aureus* and Streptococcus spp. was done thoroughly. Based on the results of the study, the probiotics, live and inactive *S. cerevisiae*, caused a significant reduction of the traits of total bacterial count (*p=0.007*), *Streptococcus* sp. (*p=0.046*), *Coliform* (*p=0.041*), and *Lactobacillus* (*p=0.032*) in the intestines of the birds; only a little significant reduction (*p=0.01*) of the trait of total bacterial count was observed in the meat. The present study has provided evidence that the supplementation of probiotics in the diet of Japonica quail significantly reduced intestinal microbial flora. Thus, it may be concluded that probiotics help in reducing the microbial properties of the meat and intestines of Japonica quail.

Key words: Probiotic, *Saccharomyces cerevisiae,* microbial properties, Japonica quail.

INTRODUCTION

Recent outbreaks of food-borne diseases highlight the need for reducing bacterial pathogens in foods of animal origin. Animal enteric pathogens are a direct source of food contamination (Gaggia et al., 2010). The ban on antibiotics as growth promoters (AGPs) has been a challenge for animal nutrition, increasing 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 world agribusiness trade. The importance of feed supplementation in poultry production has increased in the last years with the aim of improving the economic 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.,

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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 related to feed additives, is limited. In the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease 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 and 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 et al., 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 et al., 2005). Probiotic is a relatively new word meaning 'for life', which is used for naming microorganisms that are associated with beneficial effects for humans and animals. These microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic micro-organisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus, Pediococcus* and some yeast have also been found as suitable candidates. Together they play an important role in the protection of the organism against harmful microorganisms and also strengthen the host's immune system. Probiotics can be found in dairy and non dairy products. They are usually consumed after the antibiotic therapy (for some illnesses), which destroys 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 (RicardoSocco 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 differing areas of adherence (site-specific), specific immunological effects, and actions on a healthy *vs.* an inflamed mucosal milieu 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 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 between 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 can be cited 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 to human and animal health. Much 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, colonise and modulate the human gastrointestinal system is not a universal property. *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 other important properties. Besides, the consumption of dairy and nondairy products, it 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

Table 1. Ingredient of basal diet.

Ingredient	%
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

were used (Saadia and Nagla, 2010). Therefore the aim of this study was to evaluate the effects of probiotic (Live and inactive *Saccharomyces cerevisiae)* on meat and intestinal microbial properties of Japonica quail.

MATERIALS AND METHODS

Birds and housing

24 Japonica quail aged 1 day were obtained from a commercial hatchery. The birds were randomly divided into 2 groups (12 birds/group) and housed in pens of identical sizes (1.75 × 6 m) in a deep litter system with a wood shaving floor. Each of the birds 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 Japonica quail were fed with the diets from day 1 to day 72. Basal diet of control group has been shown (Table 1).

Meat and intestinal samples preparation

At the end of the experiment 12 Japonica quail 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 was adjustely weighed and transferred into test tube containing 9 ml of 0.1 sterile peptone and the samples were properly mixed and serial dilutions were prepared.

Enumeration of bacteria

Collected meat and intestinal samples were transported at 4°C to a laboratory of food hygiene in Islamic Azad University Tabriz Branch. In this study of each samples, 25 g was prepared according to Standard methods of Institute of standards and Industrial Research of Iran; No: 356, 1810, 2197, 1194 and 437 for preparation, culture and detection of Bacterial total count, Coliforms bacteria, *Clostridium perfringens, S. aureus* and *Streptococcus* spp and for enumeration of Lactobacilli bacteria according to method of Saadia and Nagla, was done (ISIRI 1981a, b 1992,1993, 2006; Saadia and Nagla, 2010).

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):

1. According to results related to effects of Probiotic (Live and inactive *S. cerevisiae) on* intestinal microbial properties of Japonica quail: the probiotic cases have 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)*, but a significant effect on *S. aureus* and *C. perfringens* properties were not observed (Table 2).
2. With reference to results of the effects of Probiotic (Live and inactive *S. cerevisiae) on* meat microbial properties of Japonica quail: only significant reduction on properties of *Total bacterial count* was observed *(p=0.01)* and significant effect on reducing the properties of *Streptococcus sp.*, *Coliform*, *Lactobacillus*, *S. aureus* while *C. perfringens* were not observed (Table 3).

Probiotic strains have been shown to inhibit pathogenic bacteria both *in vitro* and *in vivo* through several different mechanisms. The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora through 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 (Ayasan et al., 2006). 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 conventionally fed groups at the 2nd, 4th and 6th week of age. The result revealed competitive antagonism. The result of their study also evidenced that probiotic organisms inhibited some nonbeneficial 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 impetus 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

Table 2. Effects of probiotic (Live and inactive *Saccharomyces cerevisiae)* on intestinal microbial properties of Japonica quail.

Bacterial	Groups						Sig
	Control			Probiotic			
	N	Mean	SD	N	Mean	SD	
Total bacterial count	12	8.2109	1.16991	12	5.6959	0.39534	0.007
S. aureus	12	2.2470	1.78261	12	0.7964	1.36970	0.402
F. Streptococcus	12	2.9075	2.6669	12	0.8731	1.3496	0.046
C. perfringens	12	4.7026	1.48716	12	3.3873	1.6398	0.292
F. 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

Table 3. Effects of probiotic (Live and inactive *S. cerevisiae)* on meat microbial properties of Japonica quail.

Bacterial	Groups						Sig
	Control			Probiotic			
	N	Mean	SD	N	Mean	SD	
Total bacterial count	12	3.8594	0.4289	12	2.7968	0.6555	0.01
S. aureus	12	2.0261	0.5284	12	1.6667	0.4211	0.835
F. streptococcus	12	5.2761	1.1648	12	4.9114	1.1298	0.947
C. perfringens	12	1.9262	1.7535	12	1.7052	0.9984	1.00
F. 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

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 week 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).

In a study by Sadiaa et al. (2010) results show 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 also reveals that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of growth, modulation of intestinal microflora and pathogen inhibition, immunomodulation and promoting meat quality of poultry (Saadia and Nagla, 2010). 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 binding specific 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. Results have shown that yeast does not permanently colonize animals, the yeast and any yeast-bound pathogens are passed out in the bird 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 a 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 a suppression of pathogenic species. Numerous factors such as animal to animal variation, strain of yeast, and experimental procedures have contributed to the variation in 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 yeast culture supplementation (Kabir et al., 2004).

In a study by Pelicano et al. (2003) 150 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 breast muscle 5 hours 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 fed in the water and diet instead of only in the diet. Nevertheless, carcass and meat quality showed no alteration when the control group was compared to birds fed with probiotics, except for leg yield improvement in the latter (Pelicano et al., 2003).

Conclusion

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

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