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Synbiotics as potentially growth promoter substitution for improving microbial and oxidative stability of Japanese quail meat

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Abstract

The use of antibiotics in livestock breeding, especially poultry, leads to an increase in antibiotic resistance and human disorders. Therefore, researchers are seeking a good substitute to improve gut microbial balance, growth performance, and meat quality of livestock. The present study was aimed to investigate the effect of diets containing different levels (0, 90, and 100%) of probiotic Fermacto (F), prebiotic Primalac (P), and their mixture on chemical, microbial, and sensory properties of Japanese quail meat. The F_{100} sample showed the highest color and odor scores; whilst, juicier feature was more dependent on prebiotic level. $F_{100}P_{100}$ and $F_{90}P_{90}$ ranked the highest meat flavor and overall acceptance scores, respectively. The lowest number of microorganisms and total coliforms were observed in $F_{90}P_{100}$ during storage. In general, the addition of synbiotics to the diet of Japanese quail led to improve in meat quality and decrease in microbial contamination besides controlled oxidation during refrigeration.

Keywords: Japanese quail; Antibiotic; Synbiotic; Poultry; Meat quality.

Introduction

Nowadays, as a result of increasing population growth and food-borne illnesses, access to healthy and safe foods has become one of the main human concerns (Severino et al., 2015; Timmer, 2017). Protein sources such as meat play a key role in human nutrition (De Smet & Vossen, 2016). However, the prevalence of cardiovascular diseases and diabetes in today's societies caused by eating unhealthy foods such as red meat has increased the demand for white meat (Bronzato & Durante, 2017; Khademipoor et al., 2017).

Poultry meat in comparison to other domestic animals has only 3.5 to 5% fat, which mostly includes unsaturated fatty acids (Marcinčák et al., 2008). Quail is a valuable and economical bird with significantly high breeding level in many countries, which can be a potential substitute to chicken due to some

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characteristics such as rapid growth, delicacy, low feed intake, early onset of lay, high egg production, short generation and incubation periods, high resistance to many common diseases, cardiac friendly, and high quality meat and eggs (Panda et al., 2017). Poultry breeding, at an industrial scale, results in an increase in many microbial diseases and subsequently antibiotic usage. It was reported that excessive consumption of antibiotics and the presence of drug residues in poultry carcasses can threaten human health (Ashraf et al., 2018; Mehdi et al., 2018; Muaz et al., 2018). Therefore, many researches have focused on introducing antibiotic substitutes (Barbieri et al., 2015; Danka et al., 2007; Mehdi et al., 2018; Nasehi et al., 2015). In an attempt to replace antibiotics (avilamycin+ sodium monensin), the probiotics effect of (Bacillus amyloliquefaciens) and organic acids (lactic, acetic, and butyric acid), indivitually or combined, on intestinal anaerobic bacteria, allometric growth of digestive organs, intestinal morphometrics, and broiler chicken performance was studied by Barbieri et al. (2015). In another study, probiotic protexin (in place of antibiotics) was successfully used in Japanese quail feed and its effects on meat properties were examined by Nasehi et al. probiotic (2015). The controlled microorganism activity, reduced oxidation reactions, increased water-holding capacity, and improved meat color index. Probiotics and prebiotics are interesting alternatives that can improve gut microbial balance and natural defense system of animals by suppressing the growth of pathogenic bacteria (Roberfroid, 2000).

Probiotics are health-promoting microorganisms that exist in the large intestine as a natural microbiota, and can lead to a significant health effect on the host through maintaining and improving the microbial balances of the intestine, if they reach the minimum amount of 10⁷ CFU/mL. Lactobacillus and Bifidobacterium strains are the most important probiotics, having some functionalities include anticarcinogenic, antimutagenic, antiseptic activities, immune stimulation, lowering serum cholesterol, increasing nutritional value, and treating diarrhea and gastrointestinal tract infections via preventing the attachment of gastrointestinal pathogens and producing antibacterial compounds (Pandev et al., 2015: Tripathi & Giri, 2014). Prebiotics, as indigestible and bioactive compounds, do not hydrolyzed in the stomach and small intestine and selectively stimulate the growth or activity of health-promoting bacteria in colon, thereby improving the host health (Bigliardi & Galati, 2013; Gibson & Roberfroid, 1995). Synbiotics are a mixture of probiotic microorganisms and prebiotic compounds that together render more health effects (Pandey et al., 2015). To the best of our knowledge, there are very few studies on the effect of synbiotic diets based on Primalac and Fermacto on the physicochemical, sensory and microbial characteristics of Japanese quail meat. The aim of this study was therefore to investigate the effect of diets containing different levels of probiotic, prebiotic, and synbiotic on oxidative stability, microbial, and sensory properties and shelf-life extension of Japanese quail meat.

Materials and methods

Probiotic Fermacto (containing *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*, and *Enterococcus faecium*) and prebiotic Primalac (fermentation product of *Aspergillus orizae*) were provided from Star-Labs (USA) and PET-AG, Ltd (UK), respectively. Butylated hydroxyl toluene (BHT), trichloroacetic acid (TCA), and malondialdehyde were purchased form Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were analytical grade and purchased from Merck (Darmstadt, Germany).

Quail management

A total of 405 quail chicks (1-day-old) were allocated to 9 treatments with 3 replicates of 15 chicks each based on a full-factorial completely randomized design. The experimental treatments were included tree levels of 0, 90, and 100% of the recommended levels of Fermacto and Primalac for the initial and growth periods (Table 1). These supplements were added to the quail diet based on NRC (National-Research-Council, 1994). Two quails of each replicate were randomly chosen and slaughtered after 42 days of breeding. The

slaughtered quails were de-skinned, trimmed into thigh and breast muscles, and then transferred to the laboratory under cold storage. Next, the thigh muscles were manually deboned, coded, and stored in refrigerator until further analysis at 1, 4, and 7 days of storage.

Table 1- Diet formulations used in this study.		
Treatment	Fermacto+ Primalac (%)	Code
1 (Control)	0	F_0P_0
2	0+90	F_0P_{90}
3	0+ 100	F_0P_{100}
4	90+0	$F_{90}P_0$
5	90+90	F90P90
6	90+100	$F_{90}P_{100}$
7	100+0	$F_{100}P_{0}$
8	100+ 90	$F_{100}P_{90}$
9	100+100	$F_{100}P_{100}$

pH measurement

The pH of quail meats was measured based on the method of Brannan (Brannan, 2009). Briefly, 5 g of sample was mixed with 50 mL of distilled water and homogenized in a blender. Afterwards, the pH of homogenized dispersion was read at room temperature using a pH-meter (Metrohm, 827 pH lab, Switzerland).

Water holding capacity (WHC)

The quail thighs were grinded in a laboratory blender for 5 sec to create a homogeneous paste under sterile conditions. One g of the obtained paste was completely covered with a Whatman No 1 filter paper and then centrifuged at 1400 rpm for 4 min. The meat sample was separated from filter paper and weighed. Afterwards, the sample was dried in an oven at 90°C for 5 min and then re-weighed. Finally, WHC of samples was calculated according to the following equation (Nasehi et al., 2015):

WHC (%) =
$$[(A_1 - A_2)/A_0] \times 100$$
 (1)

Where, A_0 , A_1 , and A_2 are the weight of sample before centrifugation, after centrifugation, and after drying, respectively.

Oxidative stability

One g of the paste of quail thighs was mixed with 2.5 mL of 0.8% butylated hydroxyl toluene (BHT) and 4 mL of 5% trichloroacetic acid (TCA) solution. The suspension was centrifuged at 3000 rpm for 3 min. The upper phase was discarded and the lower phase was reached to 5 mL with 5% TCA. 2.5 mL of the obtained solution was charged with 1.5 mL of 0.8% BHT in a screw-capped tube. Afterwards, the tube was placed in water bath at 70°C until the color change was observed (~30 min). The tube was then immediately cooled in an ice bath followed by storing it at ambient temperature. Thiobarbituric acid (TBA) values of the meat samples were spectrophotometrically measured expressed at 521 nm and as mg malondialdehyde/kg of meat (Botsoglou et al., 1994).

Sensory evaluation

The sensory properties of the cooked meat such as color, odor, juiciness, tenderness, taste, and overall acceptability were evaluated by 10 panelists based on a 7-point hedonic scale test (score 1 for dislike extremely and score 7 for like extremely).

Color evaluation

The lightness (L), redness (a), and yellowness (b) of the meat samples were measured by a Chroma meter (CR-400, Konica Minolta, Japan).

Microbiological analyses

The meat samples were pasted by a meat grinder under aseptic laboratory conditions. The serial dilutions were made by 0.1% peptone water. For coliforms enumeration in meat samples, one mL of 10⁻² dilution was poured into petri-dish using pour plate method and then charged with violet red bile agar medium. In order to determine total microbial count, 0.01 mL of each ten-fold dilution was spread on plate count agar plates. The plates were then incubated at 35- 37°C for 24 h and the results were reported as log_{10} colony forming units (CFU) per gram of meat sample $(\log CFU/g).$

Statistical analysis

Data were analyzed using a factorial experiment in a randomized complete block design with SAS software (version 9.1). Duncan's multiple range test was used to compare the means at 5% significance level $(p \le 0.05)$.

Results and discussion

Changes in pH

The metabolic process stops due to bird slaughtering and ceasing of blood flow.

processes continue for However, some moments that lead to glycogen breakdown in an anaerobic pathway and production of lactic acid, which the latter reduces pH of tissues (Asghar et al., 1991). As can be seen in Fig. 1, the highest pH value belonged to $F_{100}P_{100}$ treatment and the lowest pH belonged to F_0P_{90} on the first day of storage. Increasing probiotic content resulted in an increase in pH value of meat samples. As storage time increased to 4 days, $F_{100}P_{100}$ and $F_{100}P_0$ treatments showed the highest and lowest pH values, respectively. Also, the control sample was not significantly differed from other treatments ($p \le 0.05$). Different levels of probiotic, prebiotic, and synbiotic did not have a significant effect on meat pH at 7 days of refrigeration. The results indicated that the presence of probiotic and prebiotic in the quail diet reduced the amount of stored glycogen due to the fact that polysaccharide levels were not sufficient to undergo anaerobic degradation, lactic acid production, and finally pH decrement. On the other hand, pH increment during storage could be attributed to amino acids-deamination and ammonia rescue. Effect of a diet containing different levels of organic selenium and vitamin E (Senobar et al., 2012) and Mentha pulegium and Zataria multiflora extracts (Khademipoor et al., 2017) on the quality of Japanese quail meat did not show any significant change in meat acidity.



Fig. 1. Changes in pH of quail meat samples during cold storage. Common superscript letters on each day show significant difference between samples ($p \le 0.05$).

WHC

The effect of synbiotic diets on WHC of meat samples is given in Fig. 2. The results showed that there was no significant difference between all samples at 1 and 4 days of storage, however; most of the treatments had a higher WHC than the control. Nasehi et al. (2015) reported that the addition of probiotic protexin to the Japanese quail diet did not significantly change WHC of the samples (Nasehi et al., 2015). WHC was significantly influenced by different levels of probiotics and prebiotics at 7 days of refrigeration. $F_{90}P_0$ had the highest WHC, while $F_{100}P_{100}$ presented the lowest content. WHC of meat samples decreased as a

function of prebiotic level. It is worth noting that WHC plays an important role in meat processing industry and its lower content increases economic losses, negative effects on the technological characteristics of meat products, and decreases sensory features, especially the appearance of fresh meat (Schäfer et al., 2002). The reduction in WHC is mainly due to the myofibril shortening, pH decrement. myosin denaturation, and actomyosin formation (Senobar et al., 2012). Therefore, as the results showed, the lack of pH reduction may lead to a dry-firm-dark (DFD) meat with high WHC.



Fig. 2. Changes in water holding capacity (WHC) of quail meat samples during cold storage. Common superscript letters on each day show significant difference between samples ($p \le 0.05$).

Oxidative stability

Lipid oxidation is one of the main problems in the meat industry that leads to flavor loss and reduced nutritional value of many meat products (Senobar et al., 2012). Although, free radicals are known as pro-oxidants of lipid oxidation in meat, fat content, and fatty acid profile can also influence the lipid oxidation during meat storage (Kim et al., 2002). As shown in Fig. 3, TBA value of all samples increased as a function of storage period and this effect was more pronounced in the control sample (F₀P₀). F₀P₉₀ significantly had the highest TBA on first day of storage. The lowest TBA was observed in $F_{90}P_{100}$ treatment. After 4 days of storage, $F_{100}P_{100}$ and $F_{90}P_0$ presented the highest and lowest TBA value, respectively. Control sample showed significantly higher oxidative compounds in meat stored for 7 days and the lowest TBA was for $F_{90}P_{90}$. Lower oxidation in quail meat fed with probiotic and prebiotic could be ascribed to antioxidant compounds produced by probiotics and their storage in meat tissues. In addition, the possible reaction of malondialdehyde with muscle components such as proteins, amino acids, myosin, and formation of carbonyl compounds could induce irregular changes in oxidation

extent (Soglia et al., 2020). Nasehi et al. (2015) reported a decrease in TBA and an increase in sensory features of fresh-fried quail meat fed with probiotics (Nasehi et al., 2015).

Additionally, in another research, fat oxidation was significantly decreased in broiler chicken meat fed with rosemary powder and vitamin E (Eftekhari et al., 2010).



Fig. 3. Thiobarbituric acid (TBA) value of quail meats during cold storage. Common superscript letters on each day show significant difference between samples (*p*≤0.05).

Sensory properties

Fig. 4 shows the effects of different levels of feeding with probiotics, prebiotics, and synbiotic on sensory properties of quail meat. The color of samples was not significantly different, except for $F_{90}P_{100}$ and $F_{100}P_{90}$ which ranked lower scores among treatments. $F_{100}P_0$ received the highest score. $F_{100}P_0$ showed higher acceptance than other treatments in

terms of odor. However, it did not show any significant difference with the control sample. The juiciness was mainly related to prebiotic content and the highest score was for F_0P_{90} , but there was no significant difference between the treated and control samples. Although, there were no significant differences between treatments in terms of tenderness, the highest score was for $F_{90}P_{90}$ and $F_{100}P_{100}$.



Fig. 4. Sensory properties of quail meats after feeding with different levels of probiotic and prebiotic.



Fig. 5. L* (a), a* (b), and b* (c) indices of quail meat samples during cold storage. Common superscript letters on each day show significant difference between samples ($p \le 0.05$).

The taste acceptance of $F_{100}P_{100}$ was considerably high and other treatments had no significant difference with the control sample. The results revealed that almost all sensory characteristics improved as the level of probiotic increased. WHC increment leads to improvement of redness, juiciness, and tenderness of meat samples. As Emadzadeh et al. (2011) stated that meat juiciness plays a key role in improving meat texture and overall acceptability (Emadzadeh et al, 2011). Fig. 4 shows the overall acceptance of meat samples. Although, no significant differences were observed between treatments, $F_{90}P_{90}$ ranked the highest score. It is noteworthy that probiotics can utilize prebiotics and produce some flavors such as acetaldehyde, diethyl acetate, and

acetoin which can be stored in meat tissues and then released by cooking.

Changes in meat color

Meat lightness varied from 24.59 to 48.7 over different storage days (Fig. 5).

 L^* index of samples on 1st day, except for F_0P_{100} and $F_{90}P_0$, did not differ significantly with the control (Fig. 5a). L^* value on 4 and 7 days was not significant among treatments. The reduction in L^* value of quail meat over time could be ascribed to water reduction which resulted in surface dryness and darkness. By studying the effect of diets containing probiotics on characteristics of Japanese quail meat during storage period, Nasehi et al. (2015) observed a decrease in L^* value of samples.

 $F_{100}P_0$ and $F_{90}P_{90}$ showed significantly higher and lower a^* value on 1^{st} day, respectively (Fig. 5b). However, there was no significant difference between other treatments. In addition, quail meats fed with 100% probiotic had relatively higher a^* index (higher redness). Redness of treatments did not differ significantly at 4 days of storage. However, at 7 days, $F_{90}P_0$ presented significantly higher a^* value than other treatments. a^* value decreased as a function of storage time. It was reported that the increase in contents of antibiotic and probiotic resulted in a non-significant increase in redness compared to the control (Nasehi et al., 2015). The increase in redness of some samples than the control could be due to antioxidant activity of probiotics and prebiotics (Das & Goyal, 2015; He et al., 2015). Meat redness depends on the presence of ferric iron and its oxidation state. Lipid autoxidation and oxymyoglobin oxidation to subsequently metmyoglobin have been related to color deterioration in meat (Nieto et al., 2010). Therefore, antioxidant compounds can decrease the red color degradation via controlling the rate of oxidative reactions. The reduced rates of lipid oxidation and metmyoglobin formation in meat supplemented with antioxidants have been reported in the literature (Lauzurica et al., 2005; Nieto et al., 2010).

Evaluation of b^* value of meat samples showed that there was no significant difference between treatments at both 1 and 7 days of storage (Fig. 5c). However, b^* value at 4 days was notably higher in treatments containing 100% probiotic and especially in 90% prebiotic $(F_{100}P_{90})$ compared to other samples. b^* value of all treatments increased as refrigeration period increased. Carotenoids are responsible for yellow color of foods and an inverse relationship is observed between red color and vellow color; an increase in redness will result a decrease in yellowness (Marconi et al., 2000). Khademipoor et al. (2017) showed that the addition of Mentha pulegium or Zataria multiflora powder to Japanese quail diets does not affect their color meat (Khademipoor et al., 2017).

Microbial load

The influence of diets containing probiotic, prebiotic, and synbiotic on microbial load of quail meat is presented in Fig. 6. Results showed that total microbial count was not affected by the experimental variables on 1st day (Fig. 6a). On the contrary, significant changes were observed at 4 and 7 days of storage. It can be seen that control sample on 4th day of refrigeration had no significant difference compared to the other treatments. The highest number of microorganisms was found in treatments rich in prebiotic and prebiotic, i.e., $F_{100}P_{100}$ and the lowest microbial count was observed in F₉₀P₁₀₀ treatment. On 7th control treatment did dav. not differ significantly with other samples, except for $F_{90}P_{100}$, which had the lowest microbial count. The highest number of microorganisms was also found in probiotic-free treatments. The results of total coliforms of quail meats during storage are provided in Fig. 6b. The highest total coliform belonged to F₀P₁₀₀ on 1st day, $F_{90}P_{90}$ on 4th day, and F_0P_{90} on 7th day. In addition, F₉₀P₉₀, F₉₀P₁₀₀, and control had the lowest coliforms at 1, 4, and 7 days, respectively. Nasehi et al. (2015) showed that the addition of probiotic lowered microorganism activity in fresh meat and total bacterial count in quails fed with antibiotic or probiotic was relatively lower than that of the control sample during storage (Nasehi et al., 2015). They also claimed that probiotic protexin like virginamycin was able to control the activity of microorganisms during cold storage, but the supplements did not play an effective role in controlling coliform activity. Similarly, a significant reduction in total microbial count in Japanese quail meats fed with probiotics was reported by Javadi et al. (2012). The reduction in microbial count by probiotics could be due to several mechanisms such as maintaining natural microbiota of the intestine via eliminating competitive factors, replacing the metabolic process through increasing the activity of digestive enzymes and reducing the activity of bacterial enzymes, improving food absorption and digestion, and stimulating the immune system (Javadi et al., 2012).



Fig. 6. Total microbial count (a) and coliforms (b) of quail meat samples during cold storage. Common superscript letters on each day show significant difference between samples ($p \le 0.05$).

Probiotic microorganisms can probably produce some bactericidal and bacteriostatic compounds in intestine such as lactoferrin, lysozyme, hydrogen peroxide, and organic acids, which potentially have antimicrobial effect toward pathogenic bacteria (Kabir et al., 2005). The addition of prebiotics galactomannans, oligosaccharides, and arabinoxylans to broiler chicken diet, reduced Salmonella typhimurium in ileum and cecum Lactobacillus and increased and Bifidobacterium species in cecum (Faber et al., 2012). Some oligosaccharides, especially galactomannans may directly prevent pathogenic bacteria binding to epithelial cells in small intestine and inhibit their proliferation in the gastrointestinal tract. Moreover, Kalsum et al. (2012) stated that probiotic Lactobacillus *fermentum* can inhibit the growth of pathogenic bacteria such as *S. typhimurium* and *Escherichia coli* in quail via producing antibacterial compounds (Kalsum et al., 2012).

Conclusions

The present research shows that probiotic and prebiotic addition did not influence pH of quail meats. Moreover, most treatments non-significantly higher presented water holding capacity than the control after 4 days of storage. Quails fed with F90P100 showed the lowest meat oxidation in fresh state. However, oxidation increased as function a of refrigeration and its rate was lower in probioticrich samples. Probiotic had more positive effects on color and odor scores; whilst, meat juiciness influenced more by prebiotic addition. It was shown that $F_{100}P_{100}$ and $F_{90}P_{90}$ treatments received higher taste and overall acceptance

than others, respectively. Synbiotic effect on microorganisms of meat showed that $F_{90}P_{100}$ had lower total microbial count compared to other treatments after 4 and 7 days of cold storage. Total coliforms of meat samples affected remarkably at initial storage periods and the lowest number was accounted for F₉₀P₁₀₀ treatment. Taking everything into consideration, synbiotic addition to Japanese quail diet can improve meat quality and control oxidation along with microbial lipid contamination during cold storage. In this study, the F90P100 feeding treatment was introduced as the best treatment with highquality meat.

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سین بیو تیک ها به عنوان جایگزین بالقوه محرک رشد برای بهبود پایداری میکروبی و اکسیدشوندگی گوشت بلدرچین ژاپنی بهزاد ناصحی **- مجید نوشکام - میترا قدسی "- احمد طاطار ^ع

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چکیدہ

استفاده از آنتی بیوتیکها در پرورش دام بهویژه طیور منجر به افزایش مقاومت آنتی بیوتیکی و اختلالات انسانی می شود. بنابراین، پژوه شگران به دنبال یک جایگزین خوب برای بهبود تعادل میکروبی روده، عملکرد رشد و کیفیت گوشت دام هستند. مطالعه حاضر با هدف بررسی تأثیر جیرههای حاوی سطوح مختلف (صفر، ۹۰ و ۱۰۰ درصد) پروبیوتیک فرماکتو (F)، پری بیوتیک پریمالاک (P) و مخلوط آنها بر ویژگی های شیمیایی، میکروبی و حسی بلدرچین ژاپنی انجام شد. گوشت نمونه F100 بالاترین امتیاز رنگ و بو را نشان داد. در حالی که ویژگی آبداری به سطح پری بیوتیک وابسته تر بود F100P100 و F90P90 بهترتیب بالاترین امتیاز را در طعم گوشت و پذیرش کلی کسب کردند. کمترین تعداد میکروارگانیسم و کلی فرم کل در F90P100، طی مدت نگهداری مشاهده شد. به طور کلی افزودن سین بیوتیکها به جیره بلدرچین ژاپنی علاوه بر کنترل اکسیداسیون در یخچال منجر به بهبود کیفیت گوشت و کاهش آلودگی میکروبی شد.

واژه های کلیدی: بلدرچین ژاپنی، آنتی بیوتیک، سین بیوتیک، طیور، کیفیت گوشت.

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