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The Effect of Prebiotic Administration in the Diet at Unusual Times on Fecal Shedding of *Salmonella enteritidis* and Meat Characteristics of Broilers

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Abstract

Background: One of the most important foodborne pathogen which causes enteritis is *Salmonella enteritidis* (SE). Human cases are mostly associated with the consumption of eggs and poultry meat.

Objective: An experiment has been carried out to evaluate the impacts of a yeast product as liquid prebiotic on bacterial shedding, performance indices, and some breast meat characteristics of broiler chickens challenged with SE.

Materials and Methods: A total of 90 one-day-old male broiler chicks (Ross 308) were randomly assigned to three different groups with three replicates for each treatment. The treatments were as follows: (1) CONT: birds were not challenged, (2) SE: birds were challenged with SE and fed with a control diet without prebiotic, and (3) SE+PREB: birds were challenged with SE and fed with liquid prebiotic. The challenge with SE was performed on birds in groups 2 and 3 at 28 days of age. Performance parameters and *Salmonella* shedding were determined on days 7 and 14 post infection. Twelve birds per treatment were sampled at the end of the trial for evaluating characteristics of breast meat.

Results: The challenged birds which received prebiotic showed significantly higher body weight gain, lower feed intake, and lower SE shedding than SE group ($P<0.05$). No significant differences were seen in meat characteristics.

Conclusion: Prebiotics can have beneficial effects even if they are used in the diet at an unusual time. The supplementation of yeast product can improve some performance parameters and reduce bacterial shedding in SE challenged chicken.

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Background

Salmonella is a bacterial genus of the family *Enterobacteriaceae* comprising 2 species: *S. enterica*, *S. bongori*. *S. enterica* comprises 6 subspecies including *enterica, salamae, arizonae, diarizonae, houtenae* and *indica* with over 2500 serovars. Most of the serovars belong to the subspecies *S. enterica*. They are human foodborne pathogens with the ability to cause a variety of diseases such as enteritis or a systemic disease with intestinal colonization.[1] *Salmonella enterica serovar enteritidis* (SE) is the most common serovar isolated from humans in the world, which is mostly associated with the consumption of eggs and poultry meat.[2] SE infection in chickens does not have apparent clinical signs; nevertheless, inflammation might be seen in the digestive system, especially in caeca.[3] Needless to say that approaches to the prevention of foodborne *Salmonella* spp. infection in humans is of utmost importance in food production chain. Increasing concern for antibiotic-resistant pathogens has imposed more limitations on the use of antibiotic growth promoters in poultry industry. Therefore, the focus on alternative strategies such as prebiotics, probiotics, organic acids, and plant extracts has increased to secure poultry health and production. Prebiotics are basically defined as indigestible dietary components that selectively motivate the beneficial bacteria in the lower gut and consequently modify gastrointestinal tract microbiota.[4] Some studies have recently shown that yeast derivatives in poultry challenged with some infectious bacteria such as *Clostridium perfringens* exhibited a greater positive impact on feed intake, body weight gain, and livability. Thus, yeast cell derivatives were proposed

as a tool for controlling necrotic enteritis.[5] In addition, Jahanian and Ashnagar[6] evaluated the MOS (mannan-oligosaccharides) supplementation at the levels of 0.05%, 0.1%, 0.15% and 0.2% of diet in laying hens challenged with *E. coli*. The highest digestibility was gained in hens received a diet supplemented with 0.05% MOS. Moreover, Huff et al[7] showed that yeast extract supplementation significantly improved both the BW gain (BWG) and feed conversion ratio (FCR) of the challenged pullets. In another study, diets supplemented with a yeast product resulted in improved BW with a decrease in FCR in starter broilers under experimentally induced challenge conditions.[8] Generally, prebiotics are added to poultry diet from the first day of rearing. Prebiotics pass to distal parts of the digestive system, bind bacteria and prevent the colonization of pathogens. The question that arises here is whether prebiotics still have positive effects when they are not used in the first days of poultry production. Therefore, an experiment has been conducted to elucidate the impacts of a yeast product in liquid form on bacteria shedding, productive traits, and meat characteristics of broiler chickens challenged with SE.

Materials and Methods

Birds, Housing and Treatments

A total of 90 one-day-old male broiler chicks (Ross 308) were purchased from a local hatchery in Amol, Iran. The chicks were weighed on arrival and were randomly distributed into 3 different treatments with three replicates. The treatments were as follows: (1) CONT: birds received only basal diet 2) SE: birds were challenged with SE and fed with a control diet without prebiotic and 3) SE+PREB: birds were challenged with SE and fed with a control diet supplemented with prebiotic (0.5 mL/L of drinking water (vol/vol), Celmanax™, Arm & Hammer Animal Nutrition, USA). The treatments were summarized in Table 1. A basal corn/soy-based broiler diet was prepared (Table 2). No antibiotic growth promoters, anticoccidial, or any feed additives other than the object of the study were used. Chicks were offered feed and water ad libitum and were reared under continuous light. Celmanax™ liquid consists of a preparation of components derived from an enzymatically hydrolyzed yeast cell wall blended with yeast extract as well as the culture of *Saccharomyces cerevisiae* on a defined nutrient media. It contained

Table 1. Study Design Showing the Number of Replicates, Birds, and Challenge/Adding Schedule Throughout This Study

Treatments	Replicates	Birds/pen	SE Challenge	Adding Prebiotic
Group 1 (Control)	3	10	-	-
Group 2 (challenged with *S. enteritidis*)	3	10	d 28	-
Group 3 (challenged with *S. enteritidis* and fed with liquid prebiotic)	3	10	d 28	d 28

Table 2. The Composition of the Basal Diet

Item	Starter	Grower	Finisher
Ingredients (%)	1-10	11-24	25-42
Corn	55.4	59.2	64.5
Soybean meal	39	34	28
Vegetable oil	1.2	3	3.7
Oyster shell	1.1	1.1	1.05
Dicalcium phosphate	2	1.5	1.55
Common salt	0.3	0.35	0.35
L-lysine HCL	0.15	0.10	0.10
DL-methionine	0.25	0.15	0.15
Vitamin E	0.1	0.1	0.1
Vitamin and mineral premix	0.5	0.5	0.5
Calculated contents (%)			
ME (kcal/kg)	2851	3000	3094
Crude protein	21	19.17	17.07
Calcium	0.97	0.93	0.86
Available phosphorus	0.48	0.43	0.35
Sodium	0.16	0.17	0.17
Lysine	1.38	1.15	1.01
Methionine	0.70	0.55	0.48
Methionine + cystine	1.03	0.86	0.78

Vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 9800 IU; vitamin E, 121 IU; B12, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg.

20% dry matter including MOS, β-glucan and protein (nucleotides). The ambient temperature was initially set at 32°C by the first week and gradually decreased to 25°C by the third week and was then kept constant. The experimental protocol was approved by the Animal Care Committee of Amol University of Special Modern Technologies, Mazandaran, Iran.

Salmonella Challenge

On day 28, all birds in groups 2 and 3 were orally inoculated with 1 mL of SE solution containing 3×10^8 CFU/mL.[9] Furthermore, adding liquid prebiotic to the drinking water of birds in group 3 began on the same day.

Performance Parameters

Chicks were weighed on a pen basis on days 7 and 14 post infection (PI) to determine BWG. Feed intake within each subgroup was calculated on days 7 and 14 PI by subtracting residual feed from the offered feed. The FCR was calculated as the ratio of FI to BWG (g of feed/g of gain).

Assessment of *Salmonella* Shedding

On days 7 and 14 PI, approximately 0.5 g of freshly voided feces (from each bird) was briefly vortexed in 10 mL of peptone buffer. An aliquot of this mixture (100 µL) was incubated overnight at 37°C on XLD agar. White colonies with black centers were enumerated and primary data of bacteria count were converted to $\log_{10}$ CFU/mL before the analysis.

Slaughtering Procedures

At slaughtering age (42 days), 12 birds from each group were slaughtered. Final body weight (FBW) at the time of slaughter was recorded. Carcasses were kept in a cool chamber at 0 to 4°C, for the day after which the breast muscle of all chickens was harvested from the carcasses.

Physical Analyses of Breast Meat

The pH of breast muscle was measured using a pH meter (Jenway 3505, Staffordshire, UK) at 24 hours postmortem. The pH probe was calibrated using buffer solutions of pH 4.0 and 7.0, and the calibration was repeated. Drip and cooking losses were measured using the method described by Pastorelli et al.[10] In order to measure drip loss, the decrease in the weight of a previously weighed piece of meat at 4°C in a period of 24 hours is calculated. Cooking loss (%) is defined as the decrease in weight of the meat caused by cooking at 75°C in a water bath for one hour and then cooling the meat for 30 minutes followed by drying.

Chemical Composition of Breast Meat

Dry matter, protein, fat and ash content of all samples of breast muscle were analyzed using the guidelines provided by the Association of Analytical Chemists.[11]

Statistical Analysis

All data were analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS). Initial body weight of broilers on day 28 has been considered as a covariate to remove its variation effects and reduce the experimental error at the start of the trial. Tukey-Kramer was used to compare the means. All statements of significance were based on probability of $P<0.05$.

Results

The performance indices of chicks are summarized in Table 3. Birds supplemented with the prebiotic and challenged with SE showed higher body weight gain than challenged birds. The reduction in FCR in the prebiotic group was significant, but feed intake in post challenge period (day 28-42) significantly increased in the group that had received prebiotic in drinking water. Significant differences ($P<0.05$) in *Salmonella* fecal shedding were observed between SE and SE+PREB groups on days 35 and 42 (Table 4). The effect of dietary supplementation with liquid prebiotic on carcass composition and indices of broilers challenged with *Salmonella* is shown in Table 5. No significant differences were observed between the experimental groups ($P>0.05$) in terms of protein, fat, ash, moisture, drip loss, and cooking loss.

Discussion

In this study, prebiotic was added to the drinking water of broilers inoculated with SE and the indicators of performance, shedding, and meat properties were investigated. According to the results, BWG and feed intake were significantly ($P<0.05$) higher in the prebiotic

Table 3. Effect of a Liquid Prebiotic on Performance of Broilers Challenged with *Salmonella enteritidis*

Treatments	BWG (28-42)	FCR (28-42)	Feed intake (28-42)
CONT	1093.11[a]	2.15[a]	2351[a]
SE	757.97[b]	2.81[b]	2134[b]
SE+PREB	855.12[c]	2.58[b]	2213[c]
SEM	37.183	1.233	64.341
P value	0.0003	0.04	0.004

[a,b] Means within each column with no common superscript are significantly different ($P < 0.05$).

Table 4. Effects of a Liquid Prebiotic on Fecal Shedding of *Salmonella enterica* ($\log_{10}$ CFU) on Days 7 and 14 Post Infection (PI)

Treatments	7 PI	14 PI
CONT	0[a]	0[a]
SE	6.97[b]	7.12[b]
SE+PREB	6.12[c]	6.36[c]
SEM	0.109	0.190
P Value	0.034	0.032

[a,b] Means within each column with no common superscript are significantly different ($P < 0.05$).

Table 5. Effects of a Liquid Prebiotic on Carcass Composition and Indices of Broilers Challenged with *Salmonella enteritidis*

Treatments	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Drip loss (%)	Cooking loss (%)
CONT	21.08	8.55	0.77	70.32	20.7	35.07
SE	20.70	11.46	1.18	74.16	21.40	37.39
SE+PREB	20.49	8.75	0.98	73.28	23.74	39.39
SEM	0.522	2.241	0.079	0.416	3.147	1.901
P value	0.782	0.411	0.104	0.164	0.610	0.372

[a,b] Means within each column with no common superscript are significantly different ($P < 0.05$).

group than in the challenged group which is consistent with previous reports.[12-14] No significant differences were observed in the FCR of different treatment groups. There are controversial results for prebiotic efficacy among numerous bioassays. Some studies have reported that beneficial impacts of prebiotics in broilers feeding[15-17]; however, other studies did not find any significant influence of this additive on health and performance of broiler chickens.[18-19] This contradiction can be due to the different dosages and content of prebiotics, challenges, inoculation time, and age of the birds. The findings of the present study showed that *Salmonella* shedding was significantly reduced by prebiotic ($P<0.05$). Similar findings were reported. Cecal population of *Salmonella typhimurium* was significantly reduced by MOS supplementation in challenged chicks[20]. The diminution of *Salmonella enterica* count was also observed by Fernandez et al.[21] In another study, the use of MOS increased CD4+ and CD8+ lymphocyte counts in the ileum and cecum and reduced fecal shedding of *Salmonella* in chickens challenged with *S. enteritidis*.[22] Eric Line et al.[23] reported that after challenging the broiler chickens with *Salmonella typhimurium* and *Campylobacter jejuni* by oral gavage, the frequency of *Salmonella* colonization was significantly reduced by dried yeast; however, *Campylobacter* colonization was not significantly affected by yeast treatment. *Campylobacter* does not appear to exhibit the mannose-specific binding reaction. Some researchers have declared that prebiotics are more likely to reduce *Salmonella* shedding than probiotics. Murate et al[24] showed that probiotic and synbiotic additives did not influence the SE infection in laying hens and broilers; in contrast, prebiotics had a protective effect during the first week post infection.

Our results indicated no effect of liquid prebiotic on carcass composition and indices of broilers challenged with SE. There is insufficient information about the effects of prebiotics on chemical and physical parameters of chicken meat challenged with pathogens. Our finding is in line with results of Al-Owaimer et al[9] who reported no significant effect of probiotic on cooking loss and drip loss of broiler breast muscle challenged with *Salmonella*. Pelicano et al[25] reported that there was no difference in pH values and cooking loss of breast meat of broiler chickens fed with prebiotics. The dietary treatments with MOS did not affect pH of breast meat of turkeys.[26] Takahashi et al[27] reported that diet supplementation with probiotics and prebiotics in broilers did not have any significant effect on cooking loss of breast meat. Breast muscle is the greatest edible part and also the most valuable part of broilers carcass. Meat composition affects the nutritional value of meat and also the quality of meat products. Konca et al[26] reported that dietary supplementation with MOS did not affect dry matter, crude ash, and crude protein of breast meat of turkeys, which is in agreement with present finding.

Conclusion

In conclusion, prebiotics can have beneficial effects even if they are used in the diet at an unusual time. Supplementation of yeast product can improve some performance parameters and reduce bacterial shedding in SE challenged chicken.

Authors' Contributions

All authors contributed equally in this article, especially in design, laboratory analysis, statistical analysis, and manuscript writing.

Ethical Approval

The experiment was conducted according to the protocol approved by Animal Care Committee of Amol University of Special Modern Technologies.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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