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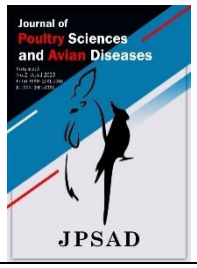

The effect of malt extract on the lactic acid bacteria (LAB) population and the *Lactobacillus* numbers in the intestine and crop of broiler chickens

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A B S T R A C T

In order to study the effect of malt extract on the lactic acid bacteria (LAB) population and the Lactobacillus numbers in the intestine and crop of broiler chickens, a total of 120 day-old broiler chicks, Ross strain were purchased and divided into four equal groups. Each group is divided into three subgroups of 10 chicks. Group A, B, and C chickens received 0.2%, 0.3%, and 0.5% of malt extract, respectively, in drinking water. Group D chickens did not get malt extract. To determine Lactobacillus and lactic acid bacteria counts, three chicks of each subgroup (9 chicks of each treatment) were randomly selected at the end of the period. One gram of the crop and ileocecal content were collected and cultured on MRS (Man–Rogosa–Sharpe agar) to determine lactic acid bacteria counts and Lactobacillus distinction, respectively. The results showed that group B, which received 0.3% malt extract, exhibited the highest lactobacillus count in the crop, indicating that this specific dosage may be optimal for enhancing lactobacillus populations and may be optimal for promoting gut health in broiler chickens. This finding is particularly noteworthy as it shows a significant increase compared to all other groups in the crop. This suggests a beneficial role for malt extract in poultry diets, supporting gut health and potentially improving overall growth performance.

Keywords: *malt extract, lactobacil counts, crop, intestine, broiler chickens.*

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1 Introduction

The application of feed additives has two objectives: controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut (1). Recently, some substances, such as phytogenic feed additives, prebiotics, and probiotics, have been used instead of antibiotics (2, 3). Herbal extracts have widely been used in food products because of their different therapeutic features. Beneficial effects of herbal extracts or active substances in animal nutrition may include stimulating appetite and feed intake, improving endogenous digestive enzyme secretion, activating immune response, and antibacterial, antiviral, antioxidant, and antihelminthic actions. Isoprene derivatives, flavonoids, glucosinolates, and other plant metabolites may affect the physiological and chemical function of the digestive tract. The stabilizing effect on intestinal microflora may be associated with intermediate nutrient metabolism (4-6). Volatile oil from thyme (*Thymus vulgaris*) was assessed for antibacterial and antiviral activity as inhibitors of microbial growth (7). In older animals, the effectiveness of plant extract supplementation was relatively low. However, higher digestibility of nutrients and reduction of *Escherichia coli* (*E. coli)* and *Clostridium spp.* in intestinal content was stated (6). Some herbs full of flavonoids, such as thyme (*Thymus vulgaris*), increase vitamin C activity, act as antioxidants, and seem to improve immune function (8, 9).

Carvacrol and thymol are the main phenolic components in *Thymus vulgaris* (8). Research has shown that vegetables, cereals, and fruits contain a very large number of phenolic compounds. Principally (80–90%), barley production is for animal feeds and malt (10). There are increasing affections in barley yields because of their high levels of phenolic acids (cinnamic and benzoic acid), tannins, chalcones, flavanones, proanthocyanidins, flavonols, flavones, and amino phenolic compounds (11). Malt contains different complexes from the malting process (Maillard reaction products) or from barley (phenolic compounds) (12). Due to the high levels of antioxidant content, barley, and malt are used as ingredients for functional food production. While the antioxidant activity of malt or barley has been studied, there is no publication recording the effects of malt extract from barley in drinking water on the lactic acid bacteria (LAB) population and the Lactobacillus numbers in the intestine and crop of broiler chickens. In the present survey, we intend to determine the effects of barley malt extract in drinking water and examine the effects of malt extract from barley in drinking water on the lactic acid bacteria (LAB) population and the Lactobacillus numbers in intestine and crop of broiler chickens.

2 Materials and Methods

2.1 Malt extract

Malt extract from barely was acquired commercially as the solution from Gorgan Malt Zarrin Co. (Golestan province, Iran).

Table 1. Chemical analysis of malt extract from barley (Gorgan Malt Zarrin Co.)

pH	3.8-4.2
Water soluble solid substances %(Brix)	60
Reducing sugars (maltos)%	At least 45
Acidity (acid lactic)	0.6
Crude Protein (%)	1.5
Moisture (%)	38
Total solid substances (%)	62
Specific Weight at 20 degrees	1.3
Refractive index at 20 degrees	1.4

2.2 Experimental design

120 one-day-old broilers, Ross strain, were purchased and housed in cages separately in the animal research unit of Shahid Chamran University of Ahvaz and received feed and water ad libitum during the experiment. The birds were reared under conditions similar to that of a one-day-old to a 47-day-old. The birds were divided into four equal groups. Each group was divided into three subgroups of 10 chicks. Groups A, B, and C received 0.2%, 0.3%, and 0.5% of malt extract, respectively, in drinking water. Group D did not get malt extract.

2.3 *Determination of Lactobacillus and lactic acid bacteria counts in intestine and crop*

For determination of *Lactobacillus* and lactic acid bacteria counts, at the end of the period, three chicks of each subgroup (9 chicks of each treatment) were randomly chosen. The contents of the distal part of the small intestine (10 cm anterior to the junction with the caecum and rectum) and crop were separately collected and used for microbial assays. The populations of Lactobacillus and lactic acid bacteria were estimated as CFU g^{-1}. Sterilized phosphate-buffered saline (PBS) (9 mL) was added to 1 g of fresh materials (1:10), and then subsequent dilutions were prepared. Fifty microliters of each dilution were cultured on MRS at 37°C for 48 hours under microaerophilic conditions, and the presence of bacteria was then determined.

2.4 *Statistical analysis*

The data were submitted for Analysis of Variance using the Statistical Package for the Social Sciences (SPSS) version 18.0. Mean differences among treatments were evaluated by One Way- ANOVA, LSD Post-Hoc Test at $p \leq 0.05$.

3 Results and Discussion

According to Table 2, the results of this study showed that the administration of malt extract did not exhibit any statistically significant effect on the lactic acid bacteria (LAB) population in ileocecal compared to the control group. Also, there were no statistically significant differences between groups A, B, and C in the lactic acid bacteria (LAB) population in ileocecal.

Also, the administration of malt extract did not exhibit any statistically significant effect on the lactic acid bacteria (LAB) population in the crop compared to the control group. There were no statistically significant differences between groups A, B, and C in the lactic acid bacteria (LAB) population in the crop.

The data presented in Table 2 indicate that the administration of malt extract did not result in statistically significant differences in LAB populations in the ileocecal and crop compared to the control group. This suggests that the malt extract, at the concentrations studied, may not have a substantial impact on enhancing LAB populations in these specific gut regions of broiler chickens, so malt extract, at the tested concentrations, does not effectively promote the growth of LAB in the broiler chickens.

Table 2. The effect of malt extract on lactic acid bacteria (LAB) population in ileocecal and crop of broiler chickens in MRS Aga

Medium groups	MRS ileocecal	MRS crop
A (0.2%)	272.9±39	247.69±32
B (0.3%)	293±41	281.75±56
C (0.5%)	232± 81	295.35± 60
D (control)	260±57.2	248.6±32

* CFU/g± standard deviation (SD) of means

Table 3 shows significant differences in lactobacillus counts between the treatment groups (A, B, C) and the control group (D) in the ileo-cecum. This suggests that the administration of malt extract at concentrations of 0.2%, 0.3%, and 0.5% positively influences the growth of Lactobacillus in the intestines compared to the control group. Also, no statistically significant differences exist between groups A, B, and C in Lactobacillus counts in the ileo-cecum.

There are significant differences between groups B and A, C, and D in Lactobacillus counts in the crop. However, there are no statistically significant differences between groups A, C, and D in Lactobacillus counts in the crop. Group B, which received 0.3% malt extract, exhibited the highest lactobacillus count in the crop (214.35 ± 11.2 CFU/g), indicating that this specific dosage may be optimal for enhancing lactobacillus populations and may be optimal for promoting gut health in broiler chickens. This finding is particularly noteworthy as it shows a significant increase compared to all other groups in the crop.

Table 3. The effect of malt extract on Lactobacillus counts in ileo-cecum and crop of broiler chickens in MRS Agar

Medium Groups	MRS ileo-cecum	MRS crop
A (0.2%)	146.4±4.5^{d}	141.4±6.6^{b}
B (0.3%)	175.9±29^{d}	214.35±11.2^{acd}
C (0.5%)	199.4±23^{d}	87.2±6.1^{b}
D (control)	81.3± 14.6^{abc}	76± 11^{b}

* CFU/g± standard deviation (SD) of means

Columns with heterogenous letters (a, b, c, and d) are significantly different ($p \leq 0.05$).

The results highlight that the 0.3% malt extract group not only showed a significant increase in Lactobacillus counts in the ileo-cecum compared to the control but also outperformed other concentrations (0.2% and 0.5%) in promoting Lactobacillus growth in the crop. This indicates that 0.3% may be the optimal dosage for enhancing gut health in broiler chickens. Significant differences were noted between group B (0.3% malt extract) and groups A, C, and D in the crop's Lactobacillus counts. This reinforces the idea that 0.3% malt extract is particularly effective in improving beneficial bacteria levels in this part of the digestive system. The findings suggest that supplementing broiler diets with malt extract at the right concentration can effectively promote the growth of Lactobacillus, which is beneficial for gut health. Increased Lactobacillus counts are associated with improved digestive efficiency and can help inhibit pathogenic bacteria. The data analysis in Table 3 illustrates that malt extract, particularly at a concentration of 0.3%, significantly enhances lactobacillus counts in both the ileo-cecum and crop of broiler chickens compared to control and other treatment groups. This suggests a beneficial role for malt extract in poultry diets, supporting gut health and improving overall growth performance.

Malt extract contains phenolic compounds that exhibit antioxidant properties (11). These antioxidants can help reduce oxidative stress in the gut, a common factor in gastrointestinal disorders. By protecting gut cells from oxidative damage, these phenolic compounds help maintain a healthy gut environment conducive to the growth of beneficial bacteria, including Lactobacillus.

The selective enrichment of Lactobacillus through malt extract supplementation helps maintain the balance of gut microflora. This balance is crucial for optimizing digestion, nutrient absorption, and immune function. Without malt extract, the gut microbiota might be dominated by harmful or less beneficial bacteria, leading to gut dysbiosis and impairing health and productivity.

In agreement with our results, Tschirch showed that Carvacrol (the thyme essential oil component) stimulated Lactobacillus proliferation (13). Also, in agreement with our results, Jamroz et al. reported that plant extract supplements also significantly increase Lactobacillus numbers, and they showed that a significant reduction of *E. coli* numbers has been obtained following an application of natural plant extract (14).

Savage et al. suggested that supplementation with oligosaccharides may have a prebiotic effect through an increase in lactic acid production, thus increasing the proliferation of beneficial bacteria and reducing the presence of Gram-negative bacteria (15).

In the present study, the administration of malt extract did not exhibit any statistically significant effect on the lactic acid bacteria (LAB) population in ileo-cecum and the crop of broiler chickens in MRS agar compared to the control group. In contrast, Rahimi et al. suggested that the lactic acid bacteria counts in the thyme group increased compared to the control group (16).

It has been documented that garlic extracts exert a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (17).

In disagreement with our results, Sedghi et al. suggested that ceca microflora enumeration did not differ among the dietary treatments of barley malt extract and malt vinegar (18).

4 Conclusions

This study showed that the administration of malt extract could increase lactobacillus counts in the intestine compared to the control group. Also, administration of malt extract at

0.3% could increase lactobacilli counts of the crop compared to all groups. The study suggests that malt extract, particularly when added to the drinking water of poultry chicks, serves as a prebiotic that selectively supports the growth of beneficial bacteria like Lactobacillus. This has several positive effects on gut health, such as enhancing the gut's ability to suppress harmful bacteria, improving nutrient absorption, and contributing to overall poultry productivity. Furthermore, the antioxidant properties of malt extract may also promote a healthy gut environment, which is essential for maintaining a balanced and productive microbiota.

Thus, malt extract is a promising tool for improving gut health in poultry and potentially reducing the reliance on antibiotics in poultry production.

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Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

F. T. and K. H. were responsible for formulating the project, developing critical conceptual ideas, and handling most technical aspects. F. T. authored the manuscript through collaborative discussions and edited it.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

I declare that I have respected all ethical standards in preparing this article. The Shahid Chamran University of Ahvaz Ethical Commission for Animal Experiments granted ethical permission.

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