



EFFECT OF ADDING SUMAC AND, RED PIPPER POWDER AND THEIR MIXTURE TO THE DIET ON SOME IMMUNOLOGICAL TRIATS AND THE MICROBIAL ACTIVITY OF THE BROILER GUT

Thamer H. Musa ¹, Bushra S. R. Zangana ²

¹Animal Production Department, Planning and Follow-up Directorate, Ministry of Agriculture, Baghdad, Iraq, thamirhassanmusa70@gmail.com

Professor Ph D., Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq, bushra.s@coagri.uobaghdad.edu

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ABSTRACT

The aim of this study was to determine the effects of adding sumac powder, red pepper, and their mixture to feed on immune traits and the microbial community of the digestive tract of broiler chickens that are raised for 42d. Two hundred non-sexed chicks of the Ross 308 breed were used. The chicks had an initial weight of 37 g per chick. They were randomly distributed among per each coop. The experiment included four treatments with five replicates each, with an average of 10 chicks per replicate. During the experimental period, the chicks were fed three types of balanced feed containing protein and energy. While the control treatment (T1) consisted of a standard feed without any additions, Add 5 g/kg of Sumac powder for the second treatment (T2) and 5 g/kg of hot red pepper for the third treatment (T3). For the fourth treatment (T4), add a mixture of sumac powder and hot red pepper, with 2.5 g/kg of each. The current study concluded that broiler chickens fed with feed containing sumac powder and red pepper achieved a higher relative weight of fabri's cecropia and a relative index of Fabri's cecropia in favor of the additive treatments. Moreover, they exhibited a higher rate of lactobacilli counts in the cecal region, along with a higher level of antibodies directed against Newcastle disease and infectious bursal disease.

Keywords: Antioxidants, Fabricia, Lactobacilli, Coliform bacteria.

تأثير أضافة مسحوق ثمار السماق (*Rhus coriaria*) والفلفل الاحمر (*Chilli pipper*) وخلطهما الى العلبة في بعض الصفات المناعية والنشاط المايكروبي لامعاء فروج اللحم .

ثامر حسن موسى ¹ ، بشري سعدي رسول زنكه ²

اقسم الانتاج الحيواني، دائرة التخطيط والتابعة، وزارة الزراعة، بغداد، العراق، thamirhassanmusa70@gmail.com

²الاستاذ الدكتور، قسم الانتاج الحيواني، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق، bushra.s@coagri.uobaghdad.edu.iq

الخلاصة

استهدفت الدراسة معرفة تأثير أضافة مسحوق ثمار السماق والفلفل الاحمر وخلطهما الى العلبة في بعض الصفات المناعية والمجتمع المايكروبي للقناة الهضمية لفروج اللحم والمربي لعمر 42 يوم، اذ استخدم 200 فراخ نوع (Ross 308) غير مجنس، وبمعدل وزن ابتدائي 37 غم/ وزع على الاكنان، وقد تضمنت التجربة اربع معاملات بواقع خمسة مكررات/ وبمعدل 10 فراخ/ مكرر، معاملة السيطرة T1 مكونة من علبة قياسية بدون اضافة بينما اضيف 5 غم/ كغم مسحوق السماق للمعاملة الثانية (T2) و 5 غم/ كغم فلفل الاحمر الحار للمعاملة الثالثة (T3) والمعاملة الرابعة (T4) خليط من السماق والفلفل الاحمر الحار 2.5 غم/ كغم لكل منها. يستنتج من الدراسة الحالية ان فروج اللحم المعذى على كل من مسحوق السماق والفلفل الاحمر الحار في علبة حق اعلى وزن نسبي لجرابية فابريشا وكذلك دليل نسبي لجرابية فابريشا لصالح معاملات الاضافة وكذلك اعلى معدل لاعداد بكتيريا العصيات اللبنية في منطقة الصائم مع اعلى مستوى للالضداد الموجه ضد مرض النيوكاسل والكمبورو.

الكلمات المفتاحية: مضادات اكسدة، فابريشا، بكتيريا العصيات اللبنية، بكتيريا القوالون.



INTRODUCTION

As a result of the imposed ban on the use of antibiotics and growth stimulants in the fodder industry and because of the negative effects they cause, including the emergence of resistance to microbes, and the presence of antibiotic residues in meat, which poses a threat to human health, poultry breeders have been motivated to find safe and natural alternatives to be added to the fodder, as the trend has become towards using medicinal and aromatic plants, spices and aromatic oils to achieve better production performance to meet the needs and requirements of the consumer (Onibi *et al.*, 2009; Povioic *et al.*, 2018; Hernández-Coronado *et al.*, 2019).

In addition to their use in food as food additives and flavoring materials (Zangana, & Al-Jumaili, 2010; Al-Safi & Zankana, 2022), additionally, they use preservatives for foods (Zangana *et al.*, 2015) and processed meats to limit the growth of harmful microorganisms (Smith, & Winder, 1996).

Sumac is an important plant that grows widely in the Mediterranean region and is used in folk medicine and as a food spice. It contains ascorbic acid, phenolic compounds, tannins, other amino acids and antioxidants (Fereidoonfar *et al.*, 2019). Feeding sumac to birds contributes to improving the feed conversion factor (Toghyani & Faghon, 2017), because it contains a wide range of active ingredients, organic acids, and terpenoids (Morshedloo *et al.*, 2018).

Red hot pepper is also important and are grown due to their economic importance because they contain capsaicinoids, which are responsible for their strong taste. It is also a good source of vitamins A, C, D, carotenoids, and phenolic compounds, the most important of which are flavonols, flavonoid glycosides, and hydroxycinnamic acid, which are natural antioxidants (Morales-Soto *et al.*, 2013) and have effective properties that contribute to improving the lipid profile in plasma and lowering the pH of the cecal content, thus inhibiting the growth of pathogens such as salmonella bacteria and coliform bacteria (Reda *et al.*, 2020).

As a result of moving toward the use of herbal plants and spices in poultry fodder globally as natural antioxidants that contribute to improving health and immune status, this study was conducted to determine the effect of adding sumac fruit powder and red hot pepper and their mixture to broiler fodder, on some immune characteristics and the microbial community of the digestive tract of broilers.

MATERIALS AND METHODS

This experiment was conducted in the poultry field of the Department of Animal Production at the College of Agricultural Engineering Sciences / University of Baghdad for the period from 26/10/2021 to 7/12/2021 42D. Two hundred Ross 308 unsexed chickens from the Al Rashidiya hatchery were used, the average initial weight was 37 gm/chick, and the chicks were distributed randomly among the co-ops, with dimensions of 2 m*1m per co-op. the experiment has included four treatments and 5 repetitions with an average of 10 chicks per repetition, as follows:

The treatment (T1) included the standard diet without any additives (control), the treatment (T2) consisted of a standard diet supplemented with 5 g of sumac powder/kg. The treatment (T3) consisted of a standard diet with 5 g of red hot pepper powder added/kg. The treatment (T4) consisted of a standard diet supplemented with 2.5 g of sumac powder + 2.5 g of red pepper powder / kg.



The rearing hall was equipped with the necessary number of lighting lamps, providing 23 hours of light and one hour of darkness to accustom the chicks to the dark when the power went out suddenly. Gas incubators were used to warm the chicks, and the temperature of the hall was 33 °C, the temperature was subsequently reduced by 2 °C every three days until it reached 24 °C at the age of 21 d. The hall was equipped with air outlets for a passive ventilation system to exchange the air and moderate the temperature of the hall's air whenever necessary. Circular plastic dishes were used to provide fodder during the first week of life, and then the dishes were replaced with suspended cylindrical feeders, with an adjustable height to suit the advancing age of the bird at the level of the bird's back. Inverted plastic watering pots were also placed on the floor, with a capacity of 2 liter and one watering pot for each replicate, for two weeks. After the plastic watering pots were replaced with plastic watering pots with a capacity of 5 liter, at the height of the bird's back, feeder was provided to the birds. (ad-libitum) during the entire experiment (42 d).

Four birds from each treatment were selected randomly, weighed individually, and then slaughtered after fasting for 4 h before slaughter. The animals were scalded at a temperature of 54°C for two minutes, the feathers were removed, and the process of removing the internal viscera was carried out in a precise anatomical manner from the beginning of the esophagus to the end of the cloaca according to previous method (Fletcher, 1999), fabricia cases were extracted after the connective tissue around the pouch was cut and then weighed using a sensitive scale to four decimal places, the relative weight of the pouch and the Fabricia guide were calculated according to method (Lucio & Hitchner, 1979)

The mborro virus treatment was administered at the end of the breeding period. This test is used to determine the titer of blood serum antibodies directed against the nevus that causes Newcastle disease and the nevus that causes Comburo disease by means of the enzyme-linked immunosorbent test (ELISA), as reported by (Mond, 1997) and then give very accurate details of the level of the bird's immune response. This examination was conducted in Al-Majmou'a Company laboratories in Baghdad.

The numbers of lactobacilli bacteria on mecitin resistant staph (MRS) Agar culture medium and coliform bacteria on MacCoky agar culture media in the duodenal area were estimated using the Pour-Plate Method and incubation in an anaerobic container at 37°C for 2 days for lactobacilli bacteria only and according to the methods described in (Association of Official Analytical Chemists, 1995). The ready-made statistical program (SAS, 2010) was used with a completely randomized design to analyze the data, and the differences between the treatments were tested using the Duncan (Duncan, 1955) multiple-data test to determine the significance of the differences between the means of the studied traits.



Table (1): Ingredients and nutrient composition of starter, grower and finisher diets as percentage.

Feed Material %	Start 1-14Day	Growth 14-28Day	Final 28-42Day
Yellow corn	46	50	52
Wheat	11	11.26	11
(protean% 48)Soybean meal	33	28	26
Concentrated protein **	5	5	5
Sunflower oil	3	4	4.4
Di Calcium phosphate DCP	0.7	0.5	0.4
Salt	0.1	0.1	0.1
limeston	1.2	1.14	1.1
Total	100	100	100
The calculated chemical composition***			
Metabolic Energy (Kcal/ kg feed)	3064.75	3174.86	3220.95
Crude Protein (%)	23.13	21.1	20.38
C / P ration (%)	2.7	2.6	2.6
Crude Fat (%)	5.6	6.7	7.2
Lysine (%)	1.32	1.19	1.13
Methionine + Cysteine (%)	0.88	0.83	0.81
Calcium (%)	0.98	0.89	0.85
Phosphorus (%)	0.48	0.44	0.42

The soybeans used were from an Argentinian source, with a crude protein content of 48% and 2440 kilocalories/kg of representative energy.

**The protein concentrate used is from animal resource, produced by the Dutch company (imported) Brocon. It contains 40% crude protein, 2107 kilocalories/kg protein as represented energy, 5% crude fat, 2.20% crude fiber, 5% calcium, 4.68% phosphorus, 3.85% lysine, 4.12% methionine, 4.12% methionine + cysteine, 0.42% tryptophan, 1.70% threonine. It contains a mixture of rare vitamins and minerals that provide meet the bird's.

***According to the chemical composition based on NRC 1994).

RESULTS AND DISCUSSION

Figure (1) shows the effect of adding sumac fruit powder and red pepper or their mixture to the fodder on the relative weight of fabricia case for broilers, as a significant increase ($P<0.05$) in the relative weight of Fabricia cases was observed in favor of the addition treatment (T2) and (T3) compared with the control treatment (T1) and the same two treatments, the addition

treatment (T4) of a mixture of sumac powder (0.25%) and red pepper (0.25%), which did not differ significantly from the control treatment.

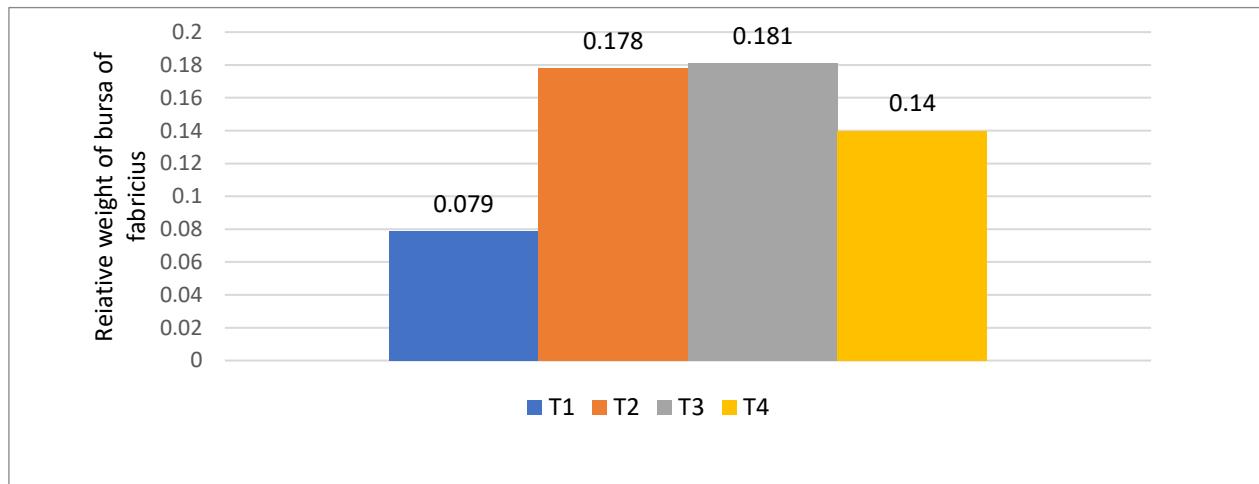


Figure (1): Effect of adding sumac fruit powder, red pepper, or their mixture to broiler rations on relative weight. For the Fabricia cas.

The Treatments include the following: T1: control, T2: 0.05% sumac, T3: 0.05% red pepper, T4: of 0.25% sumac + 0.025% red pepper. Different letters within one column indicate the presence of significant differences between the means at that level ($P<0.05$)

Figure (2) shows the effect of adding sumac powder, red pepper, or their mixture to the fodder according to the Fabrizia bursa for chickens. There was a significant increase ($P<0.05$) in the Fabrizia index in favor of treatments T2 and T3 compared to the control treatment (T1). The values for T2 and T3 were 2.25 and 2.24, respectively, compared to 1.00 for the control treatment. Treatment T4, which did not significantly differ from the control treatment (T1), had value of 1.08 compared to 1.00.

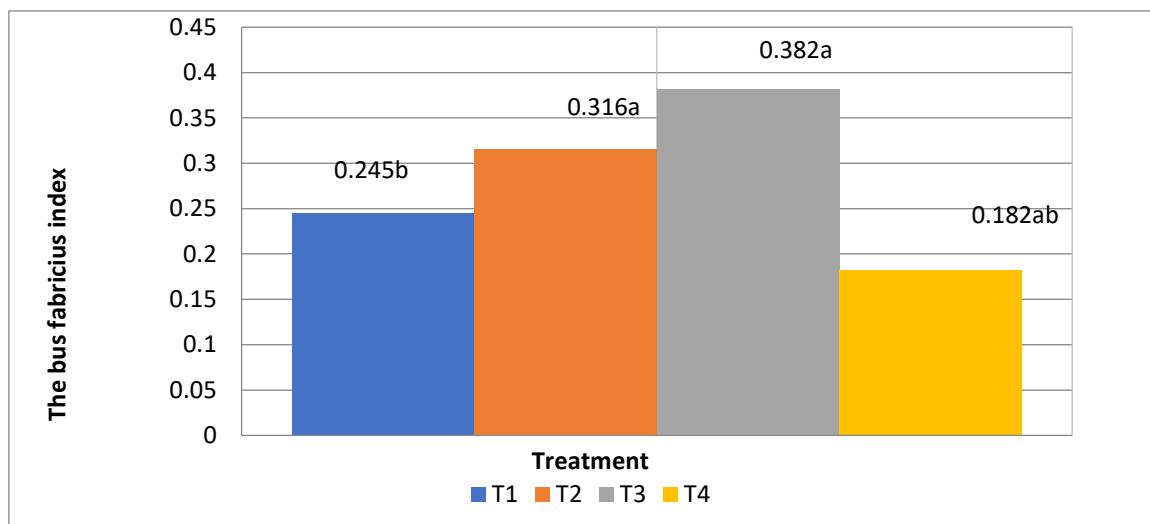


Figure (2): Effect of adding sumac fruit powder, red pepper, and their mixture to broiler rations on the relative weight of the bursa Fabricius index.

The Treatments include the following: T1: control, T2: 0.05% sumac, T3: 0.05% red pepper, and T4: 0.25% sumac + 0.025% red pepper. Different letters within one column indicate the presence of significant differences between the means at that level ($P<0.05$). Different letters indicate significant differences between the means at that level ($P<0.05$).

An increase in the relative weight of the gland of Fabricius and its index reflects the degree to which the bird immune system elevated, and indices are among the most important indicators of the extent of activity and effectiveness of the Fabricius gland. Values of the index that are less than 0.7 indicate atrophy and sluggishness of the gland (**Cummings et al., 1986; Bumstead et al., 1993**). A decrease in gland weight may be due to a low content of B lymphocytes, leading to atrophy (**Hasan, 1986; Al-Saigh, 1993**). These findings indicate that the functions of the Fabricius gland are described within the immune system, and that its size reflects bird health conditions.

After the synthesis and secretion of IgA, immunoglobulins produced by plasma cells in lymphoid tissues associated with the intestines, are transferred to epithelial cells containing a specific receptor that captures this antibody and enters the cytoplasmic gap and secretes it outside the intestinal cavity with waste without causing any pathological damage (**Nagi et al., 2012**). Figures 3 and 4 demonstrate the effect of adding sumac powder, red pepper, or their mixture to broiler fodder according to the volumetric standard against Newcastle and Gumboro viruses. There were no significant differences between the additive treatments compared to the control treatment. It was observed that birds fed on sumac and red pepper plants exhibited significantly enhanced defense systems to combat pathogens and increased activity of B cells and killer cells (**Qureshi et al., 1996**). **Al-Kassie et al (2012)** previously indicated that the addition of chili red pepper had a significant effect on increasing the L/H ratio, especially the active compound capsaicin, which contributes to supporting bird immunity and enhancing resistance to diseases.

by balancing the ratio of helper T cells and suppressor T cells and promoting the activity of natural suppressor cells. (Al-Safi, 2018) demonstrated that birds fed feed containing both cinnamon and cardamom, which contain compounds and active substances similar to those found in sumac and red pepper, significantly surpassed it in terms of antibody levels directed against Gumboro disease. This may be due to the increase in the relative weight of the gland of Fabricius and its evidence of the activity of sumac and red pepper compounds similar to interferon, which stimulates B cells to increase antibody production (Silva *et al.*, 2021). On the other hand, (Toghyani & Faghan, 2017) reported that adding sumac to feed had no significant effect on the immune antibodies of broiler chickens. These additives effectively improve the health of birds when used as nutritional supplements, whether through stimulating or inhibitory effects. They have proven their ability to improve the productive, physiological, and immune traits of birds while inhibiting pathogenic bacteria, whether inside or outside the body. All of these data have prompted scientists and poultry industry professionals to focus on these bacteria and use them in poultry feed due to their content of safe natural active substances, unlike antibiotics, which have a negative impact on public health and can cause future diseases (Wang *et al.*, 2001).

In general, all the immune response measures in this study indicated immune activity and the improved immunity in broiler chickens that consumed sumac and red pepper powder in their feed compared to those in control the treatment.

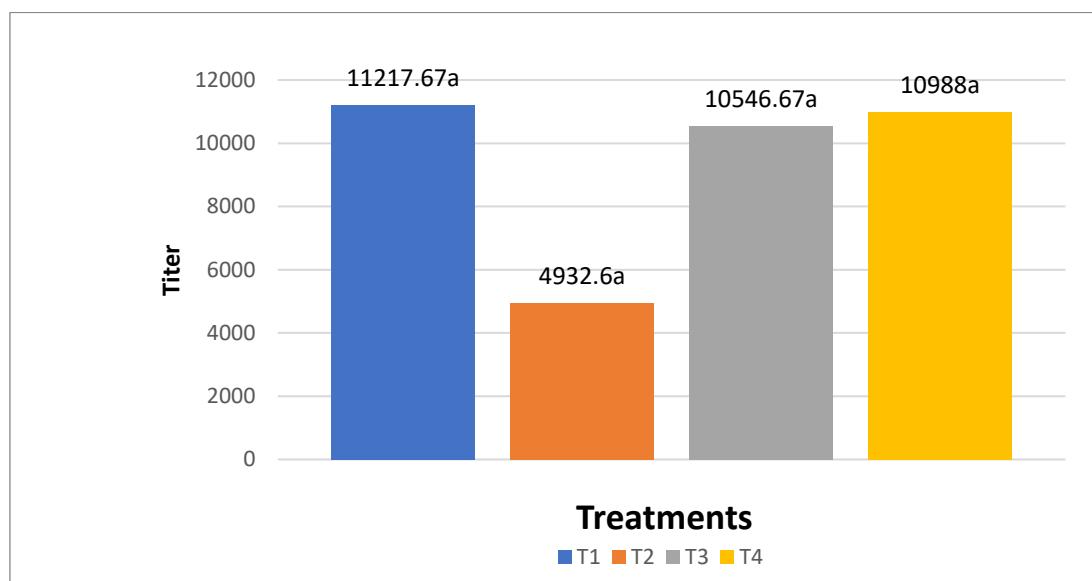


Figure (3): The effect of adding sumac fruit powder and red pepper or their mixture to the broilers at the standard volumetric level against the Newcastle virus.

The treatments include the following: T1: control, T2: 0.05% sumac, T3: 0.05% red pepper, and T4: 0.25% sumac + 0.025% red pepper. Different letters within one column indicate the presence of significant differences between the means at the level ($P < 0.05$). Different letters indicate significant differences between the means at that level ($P < 0.05$).

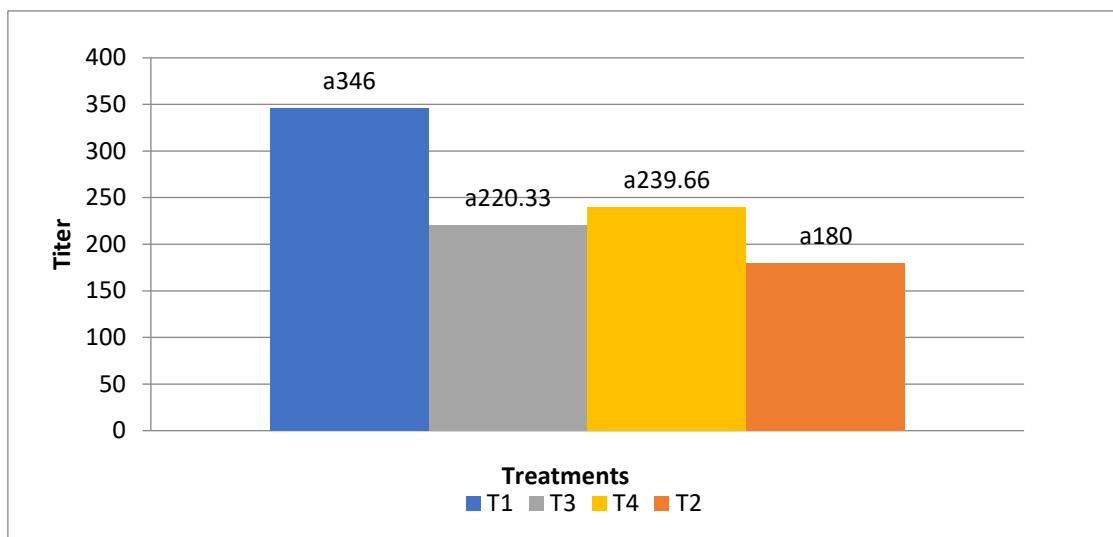


Figure (4): The effect of adding sumac fruit powder and red pepper or their mixture to the diet of broilers was evaluated according to volumetric standard against Kumboro virus.

The treatments include the following: T1: control, T2: 0.05% sumac, T3: 0.05% red pepper, and T4: 0.25% sumac + 0.025% red pepper. Different letters within one column indicate the presence of significant differences between the means at the level ($P<0.05$). Different letters indicate significant differences between the means at that level ($P<0.05$).

Figure (5) shows the effect of adding sumac and red pepper fruit powder and their mixture to the fodder on the logarithmic counts of lactobacilli and coliform bacteria during the fasting period of broiler chickens. The addition treatment T4 (0.5% sumac + red pepper) significantly increased ($P<0.05$) the number of lactobacilli, reaching $9.93 \log \text{CFU/g}$ compared to the control treatment T1, which had the lowest value of $6.86 \log \text{CFU/g}$. This was followed by the addition treatment T3 (0.5% red pepper) and then the addition treatment T2 (0.5% sumac). In contrast, there was a significant decrease in the number of coliform bacteria in favor of the T4 treatment, reaching $4.19 \log \text{CFU/g}$, followed by the T3 and T2 treatment, compared to the control treatment.

The increase in lactobacilli counts in the addition treatments may be attributed to the role of active compounds such as tannins, essential oils, phenols, ellagic acid, gallic acid, capsaicin, and quercetin present in sumac and red pepper powder. These compounds contribute to reducing the populations of harmful bacteria such as coliform bacteria (Omolo *et al.*, 2014) by inhibiting vital processes, penetrating cell walls, altering cytoplasmic permeability, and coagulating proteins. In contrast, these compounds increase the number of beneficial bacteria that secrete digestive enzymes, thereby improving the utilization of nutrients in feed and enhancing the productive performance and weight gain of birds (Dorman & Deans, 2000; Jiang *et al.*, 2007).

Our study results are consistent with the findings of (Hosseini, 2011) who reported that the antimicrobial active compounds present in the sumac diet contribute to reducing the populations of harmful bacteria in the digestive system of birds fed diets containing sumac. Similarly, (Valiollahi *et al.*, 2014) observed a significant decrease in the counts of harmful *E. coli* bacteria and an increase in the counts of beneficial *Lactobacillus* bacteria in chickens fed diets containing 0.02% sumac. These authors attributed this effect to the presence of phenolic

compounds, flavonoids, and gallic acid. Another study by (Moradi *et al.*, 2016) reported higher counts of coliform bacteria in the colon of birds in the control treatment group than in the control group. They found that Lactobacilli bacteria produce lactic acid, which is one of the end products of their fermentation process, creating an acidic environment and unfavorable conditions for the growth and colonization of harmful bacteria such as *E. coli* in the digestive tract. On the other hand, bacteria play an important role in neutralizing the negative effects of toxins and fungi through the complexes they form together, making them non-absorbable. This situation promotes the growth and proliferation of beneficial lactobacilli bacteria that are naturally present in the microbial community in the small intestine and colon (Kim *et al.*, 2009).

The results of this study are similar to those of (AL-Safi & Zangana, 2022) who reported that feeding broiler chickens raised for 42 days on diets containing 5 g of cinnamon powder or a mixture of cinnamon and cardamom (5 g/kg of fodder) similar to the composition of active substances in sumac and red pepper contributed to an increase in the number of lactobacilli bacteria and a decrease in the number of coliform bacteria in the duodenal contents compared to those in the control treatment.

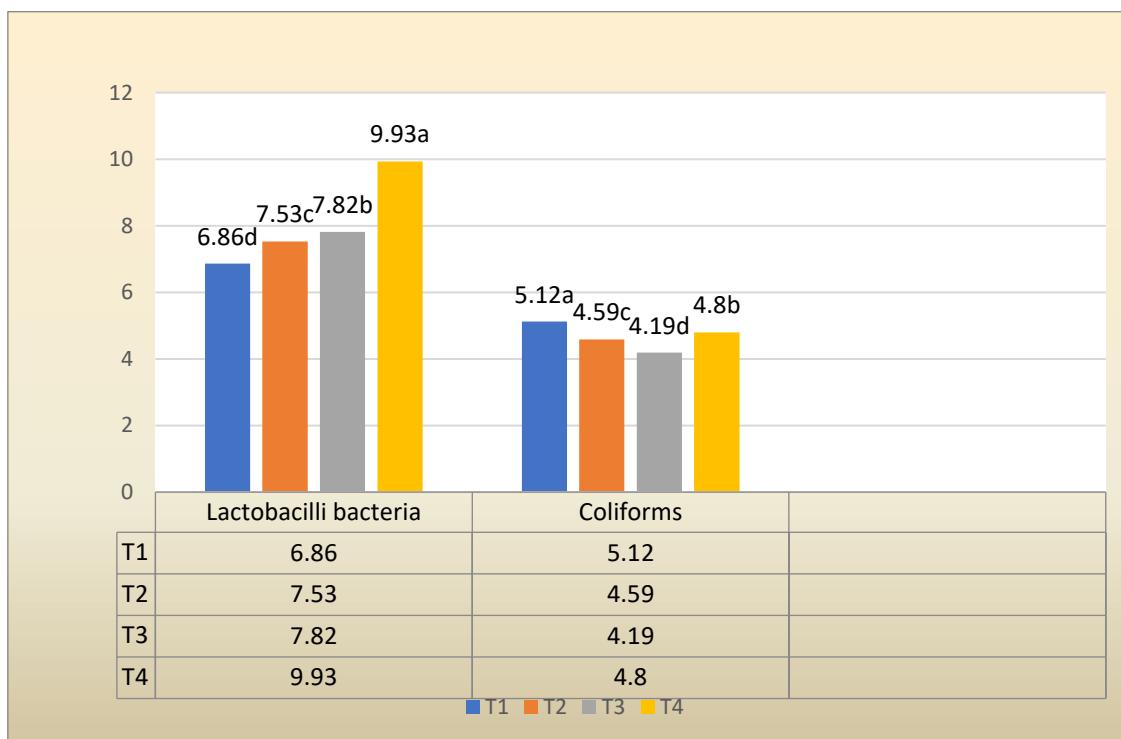


Figure (5): The effect of adding sumac fruit powder and red pepper to the feed on the logarithmic numbers of Coliform bacteria and lactobacilli (g/Log10 CFU) in the jejunal contents of broiler chickens.

The treatments include the following: T1: control, T2: 0.05% sumac, T3: 0.05% red pepper, and T4: 0.25% sumac + 0.025% red pepper. Different letters within one column indicate the presence of significant differences between the means at the level ($P<0.05$). Different letters indicate significant differences between the means at that level ($P<0.05$).



CONCLUSIONS

adding sumac powder, hot red pepper, and their mixture to the diet of poultry meat led to an increase in the population of *Lactobacillus acidophilus* bacteria in the crop region of the birds while reducing the population of colonic bacteria. This resulted in an improvement in the relative weight of *Fabrizia* and its evidence.

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