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Original Article

Evaluation of the Effect of Peppermint Extract and Probiotics on Biochemical Factors in the Blood of Ascites-Induced Chickens

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Abstract

Ascites syndrome occurs in growing broiler chickens in all parts of the world, which is one of the important causes of losses in many flocks, and its prevalence has been seen mostly in meat herds. The most important factor in the occurrence of ascites syndrome is the lack of oxygen in body tissues (hypoxia). Increasing the growth rate requires increasing the volume of blood flowing in the body to deliver nutrients to the organs and expel gases and metabolic products. Therefore, this experiment was conducted to compare the effect of peppermint extract and probiotics on the biochemical factors of the blood of chickens caused by ascites. The treatments were divided into 8 groups of 7 male chickens with 3 repetitions in each group at 21 days. The experimental treatments included control and treatments of peppermint, probiotic, peppermint and probiotic, induced ascites, induced ascites and peppermint, induced ascites and probiotics, induced ascites and peppermint and probiotics. At the end of 42 days, the blood factors of uric acid, triglyceride, glucose, cholesterol, ALT, ALP, and AST were measured by blood sampling. The experimental treatments significantly affected the investigated traits (P<0.05). Considering that in the treatment of induced ascites+ peppermint compared to the treatment of induced ascites, weight gain was significant, and in the blood factors of cholesterol, triglyceride, uric acid, glucose, and functional liver enzymes including ALT, AST, ALP recorded a significant decrease. Therefore, the effectiveness of peppermint extract in improving induced ascites in chickens was determined. Peppermint extract had a positive effect on induced ascites and improved the performance indicators of broiler chickens, and this extract can be used as a preventive of ascites.

Keywords: Peppermint extract, Induced ascites, Broiler, Probiotic

1. Introduction

One of the food sectors that can increase production by eliminating dependence on other countries and gaining currency through exports is the sector of production and supply of livestock and poultry (1). Examining the amount of chicken meat production in developed countries shows that they have a considerable income due to the importance of this food item. United States, Brazil, the European Union, and China, with 19,710, 13,800, 12,740, and 12,000 (1,000

Metric Tons) of exports, are among the largest producers of chicken meat in the world in 2018, respectively (2).

Diseases play an essential role in reducing economic power and increasing the losses of poultry breeding flocks (3). If poultry diseases are not controlled in time, they can cause irreparable damage to the poultry farmer. In recent years, losses caused by ascites in broiler chickens have been one of the world's important problems in the poultry industry. This complication has caused irreparable economic losses to broiler breeders worldwide by involving fast-growing young broiler chickens (4). In the past few years, along with the genetic and nutritional advances in the field of improving the growth rate in broiler chickens (5), the incidence of this condition has also increased dramatically. Due to the great emphasis on the growth rate in breeding programs, there is no necessary proportion between the volume and capacity of the respiratory and circulatory systems with the size of the birds, and this has caused the inability of the above systems to fully oxygenate the vital tissues of the body (6). According to the above issues, a minor change in environmental conditions, including changes in food ration, altitude above sea level, ambient temperature, ventilation, poisons, drugs, *etc*., which lead to an increase in the oxygen demand of birds, can cause heart failure and aggravate ascites (7).

Oxidative stress results from an imbalance between the production of free radicals in the body and antioxidant defence mechanisms (8). One of the most important effects of free radicals in the cell wall of aerobic organisms is lipid peroxidation. Studies have shown that the high production of oxygen free radicals (ROS), which include hydroxyl radicals, superoxide, and hydrogen peroxide, can contribute to DNA damage, protein oxidation, and lipid peroxidation in living tissues and cells (9).

Ascites syndrome occurs for three main reasons: increased pulmonary blood pressure, heart injuries, and cell damage caused by free radicals. Oxidative stress is one of the effective factors in the occurrence of pulmonary hypertension. The primary responsibility of the heart to increase tension is hypertrophy and expansion of the right ventricle; during this process, the ratio of the right ventricle to the entire ventricle (RV/TV) increases as an important anatomical index. If this ratio reaches more than 0.25, it is considered the starting point of ascites (10).

To protect against oxidative stress, living organisms have a combined antioxidant defence system that includes components of the non-enzymatic antioxidant system and the enzymatic antioxidant system. Enzymatic antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase can break free radical reactions using a chain reaction mechanism (11). Important non-enzymatic antioxidants in plasma and tissues to prevent ROS reactions include glutathione, polyphenols, carotenoids, special dipeptides, proteins containing thiol groups, polyamines, ubiquinol, flavonoids, vitamin E with selenium, vitamin C, bilirubin, and uric acid. Some of these antioxidants are produced by living organisms, while others must be supplied through the diet (12).

Achieving animal health and increasing product production and economic efficiency is one of the poultry and poultry industry's primary goals in using food supplements and antibiotics. However, due to the high consumption of antibiotics in livestock and poultry and the possibility of transferring their residues to the human body through the consumption of meat, infectious species gradually become resistant to antibiotics. Therefore, one of the important risks is the transfer of antibiotic residues to the human body through livestock and poultry and the emergence of microbial resistance. To prevent the problem, scientists suggest using medicinal plants, which have a special value and importance in biological, medical, and veterinary sciences regarding disease prevention and treatment (13). Due to the importance of these plants in medicine,

extensive research is being done to find herbal medicinal products of natural origin. In recent years, the use of medicinal plants in poultry nutrition has increased significantly. The most important reasons are proving the beneficial effects of these drugs, being cheap, having no side effects, and being environmentally friendly (13).

The Peppermint plant (*Mentha Piperita*) is a herbaceous, persistent, and perennial plant from the mint family. Among the properties of this plant, we can mention the effects of stimulating growth and immune system, as well as its anti-spasmodic and anti-inflammatory, anti-cancer, appetite-stimulating, antibacterial, and antifungal properties (14). Menthol, Mentone, and Methyl acetate are among peppermint's most essential components (15). The menthol in low concentrations selectively dilates blood vessels and causes an analgesic effect (16). It is also reported that peppermint powder had a beneficial effect by reducing the free radical activity of 2-2-diphenyl-1-picrylhydrazyl and reducing the production of malondialdehyde (MDA) compared to the control group (17). This experiment was conducted to compare the effect of peppermint extract and probiotics on the biochemical factors of the blood of chickens caused by ascites.

2. Materials and Methods

2.1. Preparation of Peppermint Plant Extract

The peppermint plant was used under standard conditions in dry shade, and the samples were used for extraction in the extraction laboratory of the Department of Pharmacy, Al-Maarif University College, Al-Anbar, Iraq. Extraction of peppermint includes grinding, pouring plant powder into labelled glass containers, adding 96% methanol alcohol to the amount of 10 times the volume of the plant, and stirring the mixture with a spoon so that the plant is well coated with alcohol and immersed in it, keep the mixture for 24 hours at a temperature of 20 ºC to dissolve the active ingredients of the plant, separate the extract solution from the plant by passing through a filter paper to an Erlenmeyer with a volume of 2000 ml, concentrate the solution containing the extract by the device Rotary, and keeping the extract in containers and the final concentration was at laboratory temperature for 48 hours.

2.2. Housing and Rearing

At first, the rearing house was separated using a cement block and a net with dimensions of 1×1 m^2 with three repetitions, and at the beginning of the breeding period, seven one-day-old chicks were randomly separated and released in each pen. In total, 168 day-old chicks of Ross 308 strain were used. Each pen had a 4-litre drinking bowl with sugar and a feeding tray. On the 21st day of the experiment, according to the end of the vaccination and the end of the resulting deaths, induction of ascites with levothyroxine and extracting by dissolving it in the drinking water of the chickens and giving the consumed probiotic as daily mixing in the feed were done. All stages of breeding, including the principles of feeding (*ad libitum*), light regime, density, room temperature, and ventilation, were carried out according to the instructions for raising broilers of the Ross breed.

2.3. Experimental Treatments

This research was conducted using 8 experimental treatments, three replicates, and 7 chicken pieces per replicate. The experimental treatments included 1) Control, 2) Peppermint extract, 3) probiotic, 4) Peppermint extract +probiotic 5) Induced ascites, 6) Induced ascites+Peppermint extract, 7) Induced ascites+probiotics, 8) Induced ascites++probiotics+ Peppermint extract. The concentration of peppermint extract, levothyroxine, and Bio-Poul was 150 ppm, 45 ppm, and 15 mg per 100 litres of drinking water, respectively. The basal rations were formulated using the requirements of the Ross breed and NRC analysis tables and by software UFFDA (Table 1).

Table 1. Ingredients and nutrients composition of the basal diet

Item (%)	1-10 days (Starter)	11-24 days (Grower)	25-42 days (Finisher)
Ingredients			
Corn	49.22	52.63	57.93
Soybean meal (440 g CP/kg)	41.66	37.87	32.39
Soybean oil	4.40	5.28	5.82
DL-methionine	0.39	0.33	0.30
L-lysine-HCl	0.21	0.14	0.14
L-threonine	0.09	0.05	0.03
Dicalcium phosphate	2.20	1.95	1.71
Calcium carbonate	1.04	0.96	0.90
Common salt	0.29	0.29	0.29
Vitamin-mineral premix[a]	0.05	0.05	0.05
Calculated composition (g/kg)			
Metabolizable energy (kcal/kg)	3000	3100	3200
Crude protein	23.00	21.50	19.50
Methionine +cysteine	1.08	1.00	0.90
Lysine	1.44	1.30	1.15
Threonine	0.97	0.88	0.79
Calcium	0.96	0.87	0.79
Available phosphorus	0.48	0.44	0.39
Analyzed composition (g/kg)[b]			
Gross energy (kcal/kg)	4040	4090	4120
Dry matter	91.59	91.50	91.30
Crude protein	22.57	21.02	18.98
Ether extract	6.86	7.80	8.44
Neutral detergent fiber	10.68	10.55	10.40
Acid detergent fiber	4.53	4.32	4.03
Ash	6.94	6.44	5.86
Calcium	1.09	1.00	0.89
Total phosphorus	0.76	0.70	0.64

[a]The vitamin-mineral premix provided the following quantities per kg of diet: vitamin A, 10,000 IU (all-trans-retinal); cholecalciferol, 2,000 IU; vitamin K3, 3.0 mg, thiamin, 1.1 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; vitamin B6, 2.94 mg; biotin, 0.5 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese (as MnSO4•H2O), 110 mg; iron (as FeSO4•7H2O), 60 mg; zinc (as ZnO), 90 mg; copper (as CuSO4), 10 mg; iodine (as Ca(IO3)2), 0.46 mg; selenium (as Na2SeO3), 0.2 mg. [b]Dry matter (method 934.01), crude protein (method 954.01), ether extract (method 920.39), ash (method 942.05), calcium (method 968.08), and phosphorus (method 965.17) were determined as per AOAC (2000) and gross energy was measured by an Adiabatic Bomb Calorimeter (Gallenkampautobomb, Leicestershire, UK). Neutral detergent fiber and acid detergent fiber were determined according to the procedures of Van Soest, Robertson (18), and sodium sulfite was used in the assay

2.4. Determination of Biochemical Serum Parameters

Cholesterol, Glucose, Uric acid, triglyceride, and liver function indicator enzymes such as ALT, ALP, and AST were measured by standard kits.

2.5. Statistical Analysis

The study was an interventional type, and a one-factor design with eight levels was used for its implementation. The data was analyzed based on one-way variance analysis, and the comparison of the mean was done using Duncan's multiple range test at the 5% level using SPSS 17.

3. Results

The results of statistical analysis showed that treatments 5, 6, 7, and 8 (numbering order from the bottom of the chart to the top) had a significant effect on weight changes compared to the control group, which means that a significant reduction was observed between the above treatments and the control group. According to the findings, the lowest amount of weight index is observed in the treatment of "induced ascites" and "induced ascites and probiotics and peppermint" treatment compared to the control group (Figure 1).

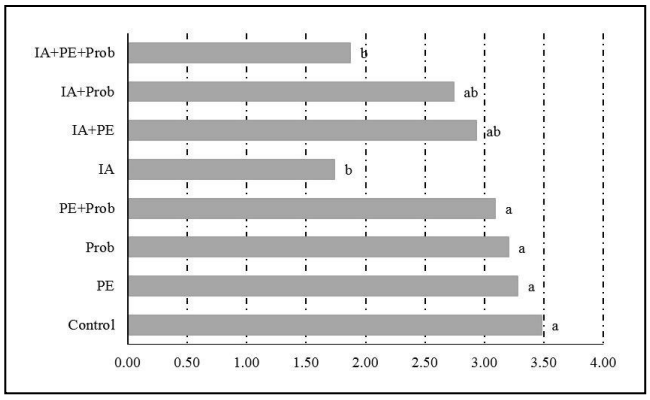

Figure 1. The effect of different treatments on weight gain
IA: Induced ascites, Prob: Probiotic, PE: Peppermint extract
Columns with different letters indicate statistical differences (P<0.05)

In this study, AST samples were measured in treatments without addition and with peppermint, probiotic, peppermint and probiotic, induced ascites, induced ascites and peppermint, induced ascites and peppermint, induced ascites and probiotics and induced ascites and probiotics and peppermint. Statistical analysis results show that treatments 4, 5, 7, and 8 showed significant differences in AST changes compared to the control group (numbering order from left to right of the chart). Based on the findings, the highest level of AST index was observed in the "induced ascites" treatment compared to the control group (Figure 2).

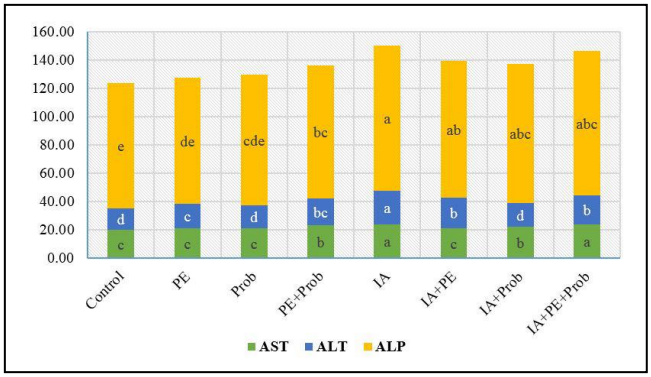

Figure 2. The effect of different treatments on liver enzymes of broiler chickens
IA: Induced ascites, Prob: Probiotic, PE: Peppermint extract
Columns with different letters indicate statistical differences (P<0.05)

In this research, ALT samples were measured in treatments without addition and with peppermint, probiotic, peppermint and probiotic, induced ascites, induced ascites and peppermint, induced ascites and peppermint, induced ascites and probiotic and induced ascites and probiotic and peppermint. Statistical analysis showed that treatments 2, 4, 5, 6, and 8 significantly affected ALT changes compared to the control group. Based on the findings, the highest level of ALT index was observed in the "induced ascites" treatment compared to the control group.

The statistical analysis results in figure 2 showed that different treatments significantly affected ALP changes. It means that a significant increase was observed between all treatments compared to the control group. Based on the findings, the highest

amount of ALP index was observed in the treatment of induced ascites compared to the control group.

Figure 3 showed that different treatments had a significant effect on glucose changes. So that a significant increase was observed between all treatments except the peppermint treatment compared to the control group. Based on the findings, the highest glucose index was observed in the treatment of "induced ascites" and "induced ascites and probiotics and peppermint" compared to the control group. With the use of experimental treatments, the highest amount of cholesterol was observed in the treatments of "induced ascites with peppermint and probiotics", "induced ascites and probiotics", and "induced ascites and probiotics and peppermint".

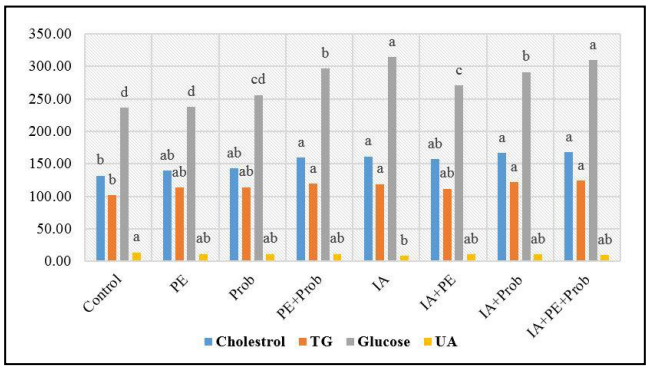

Figure 3. The effect of different treatments on some blood serum parameters of broiler chickens
IA: Induced ascites, Prob: Probiotic, PE: Peppermint extract
Columns with different letters indicate statistical differences (P<0.05)

The experimental treatments also significantly affected broiler chickens' blood serum triglyceride levels. A significant difference was observed between all treatments compared to the control group. Based on the findings, the highest amount of triglyceride index was recorded in the treatments "peppermint and probiotics", "induced ascites", "induced ascites and probiotics", and "induced ascites and probiotics and peppermint" compared to the control group (Figure 3).

Different treatments also affected the level of uric acid in the chickens' blood serum. So that there is a significant decrease in induced ascites treatment compared to the control group. Based on the findings, the lowest value of the uric acid index was observed in induced ascites treatment.

4. Discussion

The results showed that body weight and weight gain affected by ascites induction decreased significantly (P<0.05). The use of probiotics and peppermint extract led to the improvement of weight gain in birds under ascites challenge. The statistical results showed that experimental treatments significantly affected weight changes compared to the control group. In this experiment, levothyroxine was used to induce inducible ascites in chickens, and the recorded weight loss resulted from levothyroxine. The results of this experiment are consistent with the results of other researchers. Levothyroxine is the precursor of thyroid hormones, and the increase of thyroid hormones in the body causes an increase in metabolism, and the increase in metabolic energy consumption of chickens receiving levothyroxine causes their weight to decrease (19). The improvement of body weight in stressful conditions due to the use of antioxidant compounds and peppermint has been reported by several researchers (20), which is similar to the results of the present study. The researchers showed that using 0.25, 0.5, 1, and 1.5 % levels of dry peppermint in the diet of broilers leads to improved weight gain (21).

The significant difference in AST changes compared to the control group due to the use of different treatments showed that "induced ascites" showed the highest AST level compared to the control group. In some studies, it was shown that following experimental ascites with cold ascites and administration of T_3 hormone, a significant increase in ALT, AST and alkaline phosphatase enzymes was observed in chickens with ascites, and these reports are consistent with the results of this study, Abdulkarimi, Shahir (22).

A relative increase in glucose and triglycerides was observed due to ascites induction. There are reports that there is no significant difference in blood serum

glucose, triglyceride and cholesterol levels between treatment groups under stress compared to the control group, which is different from the results of the present study (23, 24). It has been reported that vitamin antioxidant compounds in inducing ascites do not affect plasma glucose and blood cholesterol levels. One study reported a significant decrease in blood glucose concentration in stress conditions compared to the control group (25). At the same time, an increase in glucose concentration in the liver of chickens with ascites was also reported. It was pointed out that most blood glucose in stress conditions is from Plasma proteins obtained through the process of gluconeogenesis. A small part of blood triglycerides participates in this process. The findings showed that under stress conditions, the amount of uric acid increases significantly; the difference in the obtained results is probably related to the type of stress, the intensity of stress, the age of the bird, the strain of the bird, etc. (26).

In general, this research showed that compounds with antioxidants have a more influential role in improving performance and peppermint extract can be used as an effective antioxidant compound in ascites conditions.

Authors' Contribution

Study concept and design: R. O. S. and S. J. A. A.
Acquisition of data: R. O. S. and S. N. A.
Analysis and interpretation of data: S. G. A. and A. Y. Y.
Drafting of the manuscript: T. A. H.
Critical revision of the manuscript for important intellectual content: Y. F. M., K. A. Z. and M. K. H.
Statistical analysis: S. G. A.
Administrative, technical, and material support: S. J. A. A.

Ethics

The study procedure was approved by the ethics committee of the Al-Maarif University College, Al-Anbar, Iraq

Conflict of Interest

The authors declare that they have no conflict of interest.

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