Effect of adding red chili pepper powder (*Capsicum Annuum*)and synthetic methionine DL-Methionine added to the feed on some physiological and immune traits of broiler chickens Ross308

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Abstract

An experiment was conducted in which 525 Ross 308 broiler chicks, one day age and with a starting weight of 43 grams, were used for a period of 35 days in which different levels of red chili pepper powder (Capsicum annuum) and the synthetic essential amino acid DL-Methionine were used to determine the extent of their effect on some physiological and immune traits. For meat chickens. These chicks were distributed randomly and equally among seven treatments that differed among themselves in terms of the nutritional treatments provided in the feed mixtures. Red chili powder and synthetic methionine were added to the chicks' diet from the first day until the age of five weeks (marketing age). The treatments were distributed as follows: First treatment (Control treatment) free of any additives. The second group was fed a diet containing 0.50 g/kg red chili powder feed. The third group of chicks was fed a diet containing 0.75 g/kg red chili pepper powder feed. The fourth group was fed a diet containing 1 gm. /kg of Red chili pepper powder feed. The fifth group fed the chicks a basic diet containing 0.50g/kg artificial methionine feed. The sixth group fed its chicks a basic diet containing 0.75g/kg artificial methionine feed. The seventh group fed the chicks a basic diet added Including synthetic methionine at an average of 1 g/kg feed. The research results showed that there was a significant superiority ($P<0.01$) for total protein in blood serum and a decrease in the concentration of glucose, cholesterol and triglycerides in the treatments to which red chili powder and industrial methionine were added compared to the control treatment. The best treatment had the highest addition (1 g/ kg of feed) whether Red chili pepper powder or synthetic methionine. The results of the study confirmed that there are significant differences ($P<0.01$) in the immune traits represented by antibodies directed against some diseases (Newcastle ND, Infectious bronchitis IBD and Comborro (IBV), as the results showed that there is a significant increase ($P<0.01$) in the rate of antibodies directed against diseases in favor of the treatments to which red chili powder and synthetic methionine were added, and that the increase in antibodies against diseases was directly proportional to the increase in red chili powder and synthetic methionine compared to the control treatment, which recorded the lowest significant value against antibodies directed against diseases

INTRODUCTION

The poultry industry has witnessed unparalleled growth over the past three decades and is now known as one of the fastest growing elements in the agricultural sector. This has happened due to the increased consumption of meat and eggs, which are rich in all essential nutrients that can meet the deficiency of important dietary minerals, vitamins and amino acids [8]. For decades, industrial antibiotics have been widely used as feed additives to treat and improve the general health of animals, as well as enhance biological functions and benefit from them in increasing growth[1] .The continuous use of industrial antibiotics as growth stimulants has

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led to The emergence of so-called bacterial resistance to these antibiotics. We can define this resistance as the ability of microorganisms to reproduce in the presence of an antibiotic agent that generally works to inhibit or kill microorganisms of the same type, resulting in the acquisition of Bacterial resistance genes [24]). This, in turn, leads to consumer fear of the emergence of bacterial strains resistant to industrial antibiotics, due to the accumulation of residues of these antibiotics in animal products. This led to a ban on the use of these antibiotics in 2006 by the European Union due to their negative impact on health. Birds and their performance due to the harm these antibiotics cause to public health resulting from the deposition of the residues of these antibiotics in poultry meat (Ravindran, 2023). Recently, consideration has been given to the use of natural alternatives to be added to the diet of poultry for the purpose of obtaining safe, high-quality foods that do not have Impact on the health of the animal or the consumer[5] . Natural plants occupy a distinguished position in the food and pharmaceutical industries for living organisms because they contain natural active compounds of great benefit and importance in their physiological effect and therapeutic activity for humans and animals. Recent years have witnessed an increasing interest in additives. Feed Natural Additives Therefore, discovering readily available alternative feed additives is necessary to protect the poultry industry in developing countries [4] . Compounds found in natural plants stimulate many biological functions in poultry, including enhancing production, improving immunity, and improving the balance of microorganisms in the intestine, which is important in regulating the metabolism and synthesis of vitamins[8] . The use of natural materials derived from plants has become widespread recently, as they are natural, free of chemical residues, stimulate digestive enzymes and produce immune bodies, and also act as antibacterial, antiviral, and antioxidants. They are relatively inexpensive and have been recognized for decades [15] Red chili pepper (*Capsicium annuum*), often known as laal mirch, is one of the most important spices used in broiler diets because it contains many active compounds. It has been used as an alternative to industrial antibiotics due to its high content of biological activities and to know the extent of its effect on physiological and immune performance [17] Adding red chili powder in high concentrations to the diet of broilers has led to an increase in the level of total protein in the blood plasma, and on the contrary, a decrease in the levels of triglycerides Which may be related to intestinal irritation or digestive disorder [4] Red chili pepper powder increases the immune response by stimulating local innate immunity and protecting against Eimeria acervulina infection and infectious bronchitis, as well as the immune response against Newcastie disease virus [13] . Synthetic methionine plays important vital roles through its ability to stimulate the immune system and raise the level of antibodies directed against Newcastle disease, increase the proliferation of lymphocytes, increase the levels of immune globulin, increase the effectiveness of the Fabricia gland and cytokines. The improvement of immunity in the bodies of broilers is also due to the presence of synthetic methionine in their system. Food that contributes to the formation of the immune system glutathione, which is a compound that carries many important elements that are most active and abundant within cells. It is an antioxidant and important

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for protection against the emergence of oxidative stress and the occurrence of inflammatory conditions[20] .

Materials and methods

This study was conducted in September 2023 AD in the poultry field affiliated with the Department of Animal Production Technologies at the College of Technology / Al-Musayyab / Al-Furat Al-Awsat Technical University on the effect of adding different levels of red chili pepper Capsicum annuum and the essential amino acid synthetic methionine DL-Methionine in some Physiological and immune traits of Ross 308 broilers. This experiment used 525 Ross 308 broiler chicks, one day age and with an average weight of 43 grams. These chicks were randomly distributed into seven treatments, each treatment containing 75 chicks, divided into three replicates, with 25 chicks in each replicate. The rearing took place in a hall equipped with the necessary rearing means. The rearing system was adopted in a semi-closed hall, and the mattress was made of sawdust and 5 cm thick. The temperature was set on the first day of rearing at 34°C, and after that it gradually decreased every week at an average of two degrees until the end of the experiment, that is, at the age of 35. One day old, the birds were vaccinated by giving the vaccine by instillation into the eye and under the skin, from a mixed type of Newcastle disease and infectious bronchitis, and at the age of 7 days, the second vaccine was given to Newcastle disease in drinking water, and at the age of 14 days, the vaccine was given to Camporo disease in water. Drinking: At 21 days old, the third vaccine for Newcastle disease was given through drinking water. The rearing period was divided into two stages, the beginning and the end. Two groups of feed mixtures were formulated so that the energy level used in the mixture was 3200 kcal/kg. The chicks were divided into seven groups. The first group (the control group) was free of any additives. The second group was fed a diet containing 0.50 g/kg. kg of red chili pepper powder feed. The third group of chicks were fed a diet containing 0.75 g/kg red chili pepper powder feed. The fourth group was fed a diet containing 1 g/kg red chili pepper powder feed. The fifth group was fed a diet containing 0.50 g/kg. kg of synthetic methionine. The sixth group was fed a diet containing 0.75 g/kg synthetic methionine feed. The seventh group of chicks were fed a diet containing 1 g/kg synthetic methionine feed. The feed was provided daily using the open feed method, and blood was collected via the brachial vein at an average of four birds from each treatment, aged 35 days, were randomly selected using 5 mm disposable syringes, and the blood was collected directly into tubes free of the anticoagulant EDTA. These tubes were placed in a centrifuge at a speed of 3000 rpm for 15 minutes, after which the serum was separated and Store it immediately at a temperature of -20°C until tests related to its biochemical and immunological traits are carried out. Physiological tests were analyzed for laboratory blood biochemical indicators, which included the determination of total protein and glucose according to the method of [27] and cholesterol according to [10] . As for triglycerides, they were estimated according to the method of [26] after which the volumetric standard for antibodies in blood serum directed against Newcastle disease, Comboro disease, and infectious bronchitis (IB) was measured using the indirect enzyme immunosorbent technique (ELISA), which is used to accurately titrate any antigen. As high as 0.0005 microml, meaning that the basis of

this test is the binding of antibodies directed against a specific antigen present in the blood serum sample to be tested, as indicated by [23,24]

After that, statistical analysis was performed using the statistical program SAS (2012) to analyze the data to study the effect of different parameters on the studied traits according to Completely Randomized Design (CRD), and the significant differences between the means were compared with the [9] test.

Results and discussion

Physiological traits

The results of the statistical analysis shown in Table (1) indicate the concentration of total protein g/dl in the blood serum of broiler chicks during the five weeks of the studied experiment. Through the results, it was noted that there was a significant difference between the experimental treatments, where the fourth treatment recorded T4 (1 g/kg). Red chili pepper) had the highest total protein concentration in blood serum compared to the control treatment and other experimental treatments, followed by the third treatment T3 (0.75 g/kg Red chili pepper feed) and then the second treatment T2 (0.50 g/kg Red chili pepper feed) respectively, Then it was followed by the seventh treatment T7 (1 g/kg methionine feed), then the sixth treatment T6 (0.75 g/kg methionine feed) and then the fifth treatment T5 (0.50 g/kg methionine feed), while the control treatment T1 recorded the lowest average total protein level in chicken blood serum compared to other experimental treatments. As for the glucose concentration mg/dl, the control treatment T1 recorded the highest rate of glucose concentration in the chickens' blood serum compared to the other experimental treatments, followed by the fifth treatment, T5, then the sixth treatment, T6, then followed by the seventh treatment, T7, and then came the second treatment, T2, and then the third treatment, T3. straight. While the fourth treatment, T4, of red chili pepper powder, recorded the lowest average level of glucose concentration in blood serum compared to the control treatment and the rest of the other treatments. The results of the table also showed that there were significant differences between the experimental treatments regarding the concentration of total cholesterol mg/dl in blood serum, as the control treatment T1, free of any feed additive, recorded the highest rate of cholesterol concentration compared to the rest of the other experimental treatments, followed by the sixth treatment, T6, then the fifth treatment, T5, and then the seventh treatment. T7 was followed by the third treatment, T3, then the fourth treatment, T4, while the second treatment, T2, for red chili pepper powder recorded the lowest cholesterol concentration in the blood serum of broiler chicks compared to the control treatment and the rest of the other treatments in the experiment. As for the concentration of triglycerides mg/dl, the control treatment T1 recorded the highest value for the concentration of triglycerides compared to the other treatments, followed by the fifth treatment T5, while no significant difference was observed between the two treatments T6 and T7, after which the third treatment came T3, followed by the fourth treatment T4, while the second treatment came. T2 has the lowest concentration of triglycerides in the blood serum of chicks compared to the other experimental treatments. The reason for the superiority of total protein in the treatment of red chili pepper powder in the fourth treatment, T4, may be due to the presence of biologically active compounds in the red chili pepper powder[2] . Adding spices and herbs, such as

red chili pepper powder, can activate enzymes involved in converting cholesterol into bile acids, thus causing a decrease in the level of cholesterol and triglycerides in the bird's blood, which in turn improves the body's fat status[19] . The presence of capsaicin in red chili pepper powder, which has a significant effect on reducing blood cholesterol levels, and the reason for this decrease may also be due to its role in stimulating the liver cholesterol enzyme 7-hydroxylase, which is necessary to convert cholesterol into bile acids and thus depletes blood cholesterol levels[3] . Polyphenolic compounds work to reduce triglycerides and cholesterol because they contain multiple phenolic compounds that prevent the oxidation of cholesterol, which leads to a decrease in the deposition of triglycerides in the blood vessels. Moreover, it prevents the oxidation of unsaturated fatty acids, and this in turn reduces the deposition of cholesterol in the blood and maintains a healthy balance. It is good for the entry and exit of fats into the blood vessels and thus has an anti-cholesterol effect [28].

Table (1) The effect of adding different levels of red chili pepper powder and synthetic methionine added to the diet on the blood biochemical traits of Ross308 broiler chicks (average ± standard error)

blood traits				treatments
Triglyceride)mg/dl(	**Cholesterol**)mg/dl(	**Glucose**)mg/dl(	**Total protein**)mg/dl(	
75.333 ±0.881 A	153.000 ±1.154 A	284.333 ±2.333 A	2.900 ±0.057 G	**T1**
47.333 ±1.201 F	113.000 ±1.732 E	117.000 ±2.081 E	4.066 ±0.088 C	**T2**
45.667 ±0.881 D	101.667 ±2.403 F	105.000 ±2.081 F	4.700 ±0.115 B	**T3**
41.333 ±0.881 E	87.333 ±1.452 G	94.000 ±2.081 G	5.766 ±0.202 A	**T4**
63.000 ±1.732 B	146.000 ±1.154 C	180.000 ±1.154 B	3.166 ±0.120 F	**T5**
53.667 ±0.881 C	140.000 ±1.154 B	168.667 ±1.763 C	3.333 ±0.088 E	**T6**
51.333 ±0.881 C	132.333 ±1.452 D	123.333 ±0.881 D	3.700 ±0.057 D	**T7**
**	**	**	**	**significant level**

** Different letters within one column mean that there are significant differences between the averages of the treatments at the level (P < 00.01): T1, control treatment, T2 (0.50 g/kg red chili

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pepper feed), T3 (0.75 g/kg red chili pepper feed), T4 (1g/kg red chili pepper feed), T5 (0.50g/kg synthetic methionine feed), T6 (0.75g/kg synthetic methionine feed), T7 (1g/kg synthetic methionine feed).

Flavonoids, alkaloids and phenolic compounds have the ability to reduce the level of triglycerides in blood serum. This can be attributed to the presence of the active chemical components of these compounds in red chili pepper powder during digestion in the intestine. Cholesterol is considered the main component of the secreted bile acids. Fibers envelop the bile acids in the intestine. They are secreted into the body, which then causes the body to withdraw cholesterol from the blood to form bile acids and thus lower cholesterol levels [30] . Synthetic methionine has other positive roles, including reducing the secretion of the hormone corticosterone to the lowest level. This leads to reducing the occurrence of the gluconeogenesis process and works to balance the level of glucose in the blood, or it stimulates the secretion of the hormone insulin from pancreatic beta cells, which leads to an increase in the entry of glucose into the tissues and maintains... Its normal level in the blood ,Also, the lower level of cholesterol concentration in the blood serum compared to the control treatment may be attributed to the reason for adding synthetic methionine in an appropriate amount to conjugate with taurine, which is the intermediate product of the metabolism of synthetic methionine with bile acids, which in turn worked to form emulsifying agents, physical breakdown of fats, reduced surface tension, and increased surface area. For fat droplets to make the lipase enzyme. The results of our current study agree with the results of the researcher [25] when he pointed out the effective role of natural additives represented by red chili powder used in broiler diets at different levels, indicating a significant decrease in the concentration of cholesterol, triglycerides, and glucose, with an increase in the concentration of total protein. In the blood during 42 days of rearing. It also agreed with what [18] found, that the increase in the concentration of total protein in the blood plasma of Ross 308 broilers, along with a significant decrease in the level of triglyceride concentrations, was due to the addition of red chili pepper powder to the diet compared to the control treatment, which had opposite results. completely. Our study did not agree with what was indicated by[7] as no significant effect was observed in the concentration level of total protein, cholesterol, triglycerides, and glucose in blood serum when different levels of red chili pepper powder were added to the diet of broiler chickens during 35 days of rearing. Compared to the control treatment. Researcher [11] . also indicated that there were no significant differences in the concentration of total protein, cholesterol, and triglycerides in the blood plasma of birds with artificial methionine added to their diets more than the requirement according to the NRC for the year 1994 at the age of 35 days of rearing compared to the control treatment, and this is not consistent with our current results.

Immune traits

Table (2) shows the effect of adding different levels of red chili pepper powder and synthetic methionine added to the feed on the level of the bulk standard of antibodies directed against Newcastle disease, bronchitis disease, and Camboro disease to Ross308 broiler chickens for a period of five weeks of the experiment. The results of the statistical analysis confirmed the presence Significant differences in antibodies to Newcastle disease,

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Camboro, and infectious bronchitis among the studied treatments. It was noted that there was a significant difference between the treatments for antibodies to Newcastle disease, as the fourth treatment T4 (1 gm/kg Red chili pepper feed) recorded an increase in the rate of antibodies against Newcastle disease compared to the rest of the experimental treatments. The other, followed by the third treatment T3 (0.75 g/kg Red chili pepper), which in turn did not differ significantly with the second treatment T2 (0.50 g/kg Red chili pepper) compared to the control treatment, and then the seventh treatment T7 (1 g/kg methionine feed), We also did not notice a significant difference between treatment T5 (0.50 g/kg methionine feed) and treatment T6 (0.75 g/kg methionine feed) respectively, while the control treatment T1 recorded a significant decrease in the rate of antibodies directed against Newcastle disease compared to the rest of the treatments , other experimental. The results of the table above also showed the size standard for antibodies directed against infectious bronchitis, as the highest rate of the size standard was reached in the fourth treatment, T4, of red chili pepper powder, compared to the control treatment and the other treatments in the experiment, followed by the third treatment, T3, then the second treatment, T2, and then came the seventh treatment, T7. No significant difference was observed between the treatments T5 and T6, while the control treatment T1 recorded the lowest rate of antibodies directed against infectious bronchitis compared to the other treatments. As for the antibodies directed against Kumboro disease, we noticed that the control treatment T1 recorded a significant decrease in the rate of antibodies directed against Kumboro disease compared to the other experimental treatments, which in turn did not differ significantly with the seventh treatment T7 for synthetic methionine, followed by the sixth treatment T6, and then the fifth treatment T5, which came It is significantly similar to the treatments T2 and T3 for red chili pepper powder, while the fourth treatment, T4, recorded a significant increase in the rate of antibodies directed against Kumboro disease compared to the control treatment and the other experimental treatments. The significant increase in the levels of standard antibodies against Newcastle disease, infectious bronchitis, and Kumboro disease in the blood serum of broilers treated with red chili pepper powder may be due to the different concentrations of red chili powder added to the feed, which may have a role. In stimulating the secretions of the immune system and the production of immunoglobulins A, M, and Y [16]. Red chili pepper powder reduces the nutritional challenges facing the components of the immune system In broilers, these include mycotoxins that reduce the number of T lymphocytes and downregulate the mRNA expression of cytokines in the small intestine[12] . Polyphenols are among the active compounds in red chili pepper powder that have an effective role in strengthening the immune response due to their multiple biological functions by binding to cellular receptors and changing signaling pathways in the cell and thus regulating the immune response in the host's body[22] . Phenols work to raise the titre of antibodies against the Newcastle disease virus. Furthermore, they have a role in increasing the stimulation of innate immunity and the immune response by increasing the number of white blood cells [29]Red chili pepper powder is rich in vitamin A, which has important properties in the

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bodies of broilers, as it is an anti-inflammatory and immune-boosting agent, and vitamin C, which is involved in the synthesis of stress hormones, and this will defend the birds' immune system and increase their resistance against diseases[14]

Table (2) The effect of adding different levels of red chili pepper powder and synthetic methionine added to the feed on the level of the bulk standard of serum antibodies against viruses for Ross308 broiler chicks (average ± standard error)

Immune traits			treatments
Campour IBV	Infectious Bronchitis Disease IBD	Newcastle ND	
424.67 ±36.996 D	3506.3 ±152.427 E	3.000 ±2.000 E	T1
818.33 ±6.089 B	4471.3 ±166.307 C	135.000 ±2.886 B	T2
820.67 ±6.009 B	4757.7 ±263.586 B	136.667 ±4.910 B	T3
1208.00 ±4.163 A	5374.7 ±127.690 A	158.000 ±7.023 A	T4
817.33 ±6.009 B	5267.0 ±94.203 D	30.000 ±7.637 D	T5
593.67 ±49.874 C	5355.0 ±93.179 D	16.667 ±6.887 D	T6
429.00 ±11.150 D	5203.3 ±56.003 D	72.667 ±4.630 C	T7
**	**	**	significant level

** Different letters within one column mean that there are significant differences between the averages of the treatments at the level (P < 00.01): T1, control treatment, T2 (0.50 g/kg red chili pepper feed), T3 (0.75 g/kg red chili pepper feed), T4 (1g/kg red chili pepper feed), T5 (0.50g/kg synthetic methionine feed), T6 (0.75g/kg synthetic methionine feed), T7 (1g/kg synthetic methionine feed).

White blood cells, macrophages, stem cells, and natural killer cells participate, which are among the basic mechanisms in the innate immune response of broilers [21] . Synthetic methionine plays important vital roles through its ability to stimulate the immune system, increase the proliferation of lymphocytes, increase the levels of immune globulin, increase the effectiveness of the Fabricia gland and cytokines. The improvement of immunity in the bodies of broilers is also due to the

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presence of synthetic methionine in their diet, which contributes to the formation of the immune glutathione. It is a compound that contains many important elements that are most active and abundant within cells. It is an antioxidant and important for protection against the emergence of oxidative stress and the occurrence of inflammatory conditions [20] Synthetic methionine improves mixture immunity, cellular immunity, and the spread of the immune response by raising the levels of white blood cells and increasing their ability to phagocytose and remove toxins[21] . Levels lower than the required level of synthetic methionine have an effect on the size of the thymus, a decrease in its cell proliferation, and a decrease in the blood serum level of the known T-cell growth factor interlukin-2, which works to reduce the immune performance of broilers [6] . The results of our study agreed with what was reported by researcher [15] when she added red chili pepper powder to feed and water on the immune response against Newcastle disease and bronchitis in broiler chickens during the rearing period. Our current study also agreed with what Lu et al. (2010) revealed that the level of vitamin A and C in Red chili peppers is six times higher than what is found in citrus fruits, and that the percentage of vitamin C in Red chili peppers is twice higher than the percentage found in green chili peppers, and that these two vitamins Important properties in the bodies of broiler chickens, as they are considered an anti-inflammatory and immune-boosting agent.

Conclusion

Adding red chili pepper powder to broiler diets in different proportions showed a positive effect on most physiological traits represented by increasing the total protein concentration and reducing the concentration of glucose, cholesterol, triglycerides, and immunity against antibodies directed at viral diseases.

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