

# Effects of Dietary Aspirin Supplementation on Growth Performance, Carcass Characteristics, Gastrointestinal Organs and Liver Enzymes, Abdominal Fats, Immune Response, Cecum Microflora and Fatty Acid Profile in Breast Meat of Broiler Chickens

Masoomeh Tavakoli

Islamic Azad University Rasht Branch

Mehrdad Bouyeh (✉ [mbouyeh@gmail.com](mailto:mbouyeh@gmail.com))

Islamic Azad University Rasht Branch

Alireza Seidavi

Islamic Azad University Rasht Branch

---

## Research Article

**Keywords:** acetylsalicylic acid, immune system, performance, blood parameters, fatty acid profile

**Posted Date:** April 28th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-435345/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

This study was performed to investigate the effect of different levels of aspirin on the diet of broilers. The experiment was conducted in a completely randomized design using 120 one-day-old male broiler chicks of commercial Ross 308 strain to study the effects of three different levels of aspirin (0, 50 and 100 mg/kg) in 3 treatments, in 4 replicates, each including 10 chicks, during 42 days. The effects of different levels of aspirin, added to a basic diet, on performance, carcass characteristics and digestive organs, blood plasma components, liver enzymes, immune system, secal microflora, and acid profile of breast meat of broiler chickens were investigated. Data analysis was performed by SAS statistical software and the comparison of the means with Duncan's multiple-range test at 5% probability level. The results showed that in the final period, the chickens fed by a diet containing 100 mg/kg of aspirin (A) had the highest feed intake and weight gain and the best feed conversion ratio compared to the other treatments. Also, according to the tables, the lowest production cost and the best European factor were related to treatment A100, which was statistically significant compared to the control ( $P < 0.05$ ). In addition, the use of the same level of aspirin resulted in a significant increase in some carcass properties and a decrease in ventricular fat compared to the control ( $P < 0.05$ ). On the other hand, the effects of using two different levels of aspirin on blood parameters and liver enzymes of broilers except for alkaline phosphatase were not significant throughout the period ( $P \geq 0.05$ ). Also, the use of the same level of aspirin had no significant effect on the function of the humoral immune system in response to antigen injection, antibody titer against sheep red blood cells (SRBC) and antibody titer against Newcastle disease and influenza virus ( $P \geq 0.05$ ). But according to the table, the highest percentage of neutrophils and eosinophils was related to this treatment. Also, aspirin treatments increased the levels of unsaturated fatty acids and decreased saturated fatty acids. Moreover, aspirin treatment led to a reduction in the population of *Escherichia coli*. So, based on the results of the present study, the use of 100 mg/kg aspirin in the diet of broilers is recommended.

## Introduction

Salicylates belong to the group of nonsteroidal anti-inflammatory drugs (Nowak., 2014). Salicylates such as sodium salicylate (SS) and acetylsalicylic acid (ASA) are used in poultry industry, due to their immune system effects, analgesic properties, and anti-inflammatory activities) (Whiting et al., 2007). However, insufficient information on tolerance levels and side effects of salicylates in poultry is not available, but Poźniak et al (2010) showed that addition of 100 and 200 mg/kg aspirin or sodium salicylate (SS) in the diet of broilers were well tolerated and showed no side effects. Acetylsalicylic acid (ASA), which is the acetylated form of salicylic acid, (Richard., 2007) is the active ingredient in aspirin (Wu et al., 2016). The benefits of aspirin include minimizing gastrointestinal, respiratory disorders, and increase of growth performance, feed use, digestion, and absorption of nutrients, and the quality of meat products in broiler chickens. In addition, aspirin is involved in lowering cholesterol and triglycerides of blood and meat and plays a role in improving immune functions and antioxidant enzymes in birds (Alagawany et al., 2017). Aspirin inhibits the biosynthesis of prostaglandins, therefore, it may be able to regulate the hypothalamus

(Truong et al., 2016). Aspirin has been shown to inhibit the enzyme cyclooxygenase, thereby inhibiting the synthesis of prostaglandins. Moreover,, aspirin inhibits the release of arachidonic acid from the sn-2 position of phospholipids by altering the activity of phospholipase A, thereby inhibiting the synthesis of prostaglandins and leukotrienes and by also inhibiting thromboxane A2 synthesis also prevents platelet aggregation. In addition, aspirin reduces the serum LDL-cholesterol by increasing the number of LDL-cholesterol receptors in different cells (Valko et al., 2005; Madamanchi., 2005). In this regard, Rokade et al (2017) had also similar findings by adding 250–500 mg/kg of acetylsalicylic acid to the diet of broilers which resulted in a significant reduction in corticosteroids and serum cholesterol ( $P < 0.001$ ). Rokade et al (2017) also reported that the use of aspirin affected feed intake and live weight gain. The FCR (feed conversion ratio), protein yield and energy efficiency all improved significantly. The relative function of thymus and bursa of Fabricius was also improved. The enzyme activity of aspartate transaminase and alanine transaminase increased significantly. In addition, aspirin usage reduced corticosteroids and serum cholesterol but only after 42 days of age. Al-Obaidi and Al-Shadeedi (2010) reported that the use of aspirin led to improved body weight gain in broilers. Madamanchi et al (2005) stated that improper environmental conditions due to increased free radicals cause oxidation and destruction of biological cells, so it can cause several disorders in intestinal tissue (Ocak et al., 2008; Sahin et al., 2013).

Therefore, antioxidants such as aspirin with effective free radical inhibition properties may eliminate the problems related to intestinal disorders caused by adverse conditions during the growing period and improve functional traits (Wang et al., 2008). Since blood metabolites and fatty acids play a direct role in human cardiovascular disease, the results of using aspirin in broilers may also be used in marketing of aspirin treated broilers.

Due to the conflicting results on the effect of antioxidant compounds on the mentioned factors and since there are limited results regarding the use of this compound in the broiler growing period, the present experiment was performed to evaluate different doses of aspirin on growth performance, carcasses characteristics and organs of the gastrointestinal tract, the components of blood plasma, liver enzymes, carcass fat, the immune system, the microbial flora of the cecum, and the profile of fatty acids in breast meat in broilers.

## Materials And Methods

This study was conducted at a broiler farm in Masal, Iran. The experiment was performed with 120 one-day-old male chickens of commercial Ross 308 strain, with a mean weight of  $45 \pm 2$  g, in a completely randomized design with 3 treatments and 4 replicates and 10 chickens per pen for 42 days. The studied treatments were treatment 1 (A0mg/kg), treatment 2 (A50mg/kg) and treatment 3 (A100mg/kg) which were used in combination with the basal diet. Aspirin was used based on the intended concentrations. The diets were ground and adjusted according to the table of nutritional needs of poultry containing the minimum recommended nutrients in the Ross 308 feed guide (Manual., 2012; Table 1). The chickens were grown in 1 m × 1 m cages on a base of cellulose rolls for 42 days. The temperature in the breeding hall was 33 degrees Celsius in the first week and then gradually decreased to 23 degrees Celsius on the

18th day of breeding and then remained constant until the end of the period. Environmental conditions were similar for all the chicks and included 23 hours of exposure and one hour of darkness, with room humidity of 65 to 70%. Access to water and food during the growing period was similar with free access. In addition, birds were vaccinated against infectious bronchitis (10th day of age), Newcastle disease (4th, 21st and 35th days of age) and infectious Bursal disease (12th day of age). All vaccines were obtained from the Razi Vaccine and Serum Institute (Karaj, Iran).

### **Growth performance and economic efficiency**

Weight gain of all the chickens of each pen in periods of 1 to 10, 11 to 24 and 25 to 42 days was calculated using a digital scale (TozinKala, Iran) with an accuracy of  $\pm 10$ g. At the end of each period (starter 1 to 10, grower 11 to 24 and finisher 25 to 42) the amount of feed remaining in each feeding device was weighed and deducted from the amount of the feed given at the beginning of each period, and as the result, the amount of the consumed feed was calculated. Feed conversion ratio was also calculated by dividing the amount of feed intake by weight gain for days 1 to 10, 11 to 24, 25 to 42, and the whole period (Sigolo *et al.*, 2019).

The following formula was used to measure the European production factor:

European production factor = Average live weight (g)  $\times$  durability percentage / feed conversion ratio  $\times$  number of breeding days  $\times 10$

The following formula was used to measure the cost of feed consumed per kilogram of live weight. The price of aspirin at the time was used, calculated separately for each diet and included in the formula.

Feed cost for each kg of live weight = (price of feed during 42 days for each chicken in terms of IRR / weight of a chicken at 42 days of age in terms of kg)

### **Carcasses characteristics, digestive organs and intestinal parts**

At the end of the experiment, after two hours of starvation, two birds of each replicate with a weight close to the mean were slaughtered and weighed using digital scales (A&D GF-300 digital scale balance, (310 g  $\times$  0.001 g, A&D Weighing Design and Manufacture, San Jose, CA)) with an accuracy of 0.01 g. Weights of featherless carcass, full body, empty body, abdominal fat, breast, thigh, wing, as well as internal organs (pancreas, heart, gizzard, spleen, bursa of Fabricius, liver, ventricular fat, duodenum, jejunum, and ileum) were measured (Shabani *et al.*, 2015).

### **Parameters of blood plasma components and liver enzymes**

At the end of the experiment (42 days), 2 birds from each pen with a weight near to the mean were randomly selected and 5 ml of blood was taken from the wing vein in order to measure blood parameters. The samples were integrated and maintained for 12 hours at room temperature and then centrifuged at 5,000 rpm for 3 minutes (Eppendorf 5702, Germany). Then, their serum was isolated and transferred to

microtubes and brought to the laboratory. After separation, the serum was kept at minus 20°C until the time of measuring blood metabolites. The serums were defrosted at room temperature and then the following: glucose, triglycerides, cholesterol, total protein, albumin, globulin, creatine kinase, lactate dehydrogenase, VLDL (Very-low-density lipoprotein), HDL (high-density lipoprotein), LDL (Low-density lipoprotein), alanine aminotransferase and alkaline phosphatase were measured. These metrics were tested with Pars Azmoon commercial kits and measured by the autoanalyzer (Hitachi 917, Japan) based on the method of Golrokh *et al* (2016).

## **Immune responses**

To investigate humoral immunity, the broiler chickens were immunized against SLBC by Lerner method (Lerner *et al.*, 1971). To prepare an SRBC injecting suspension, blood samples were taken from 3 sheep and poured into a glass containing EDTA. The globules were washed three times in PBS saline phosphate buffer and at the end 2% SRBC suspension was prepared in PBS. All the above steps were performed under sterile conditions. SRBC injection was performed on 28 and 36 days of age to 2 birds for each pen injecting 0.1 cc of the above-mentioned solution to the wing vein. The blood samples were taken 7 and 14 days after the first and second injections, on days 35 and 42 (Gore and Qureshi, 1997). Then, the antibody levels of the samples against SRBC were measured by hemagglutination method. To measure the antibody titer, special plates for microhemagglutination V were prepared. Van derzipp method was used to measure the total antibody. According to this method, to measure total anti-SRBC, 50ul of serum sample was mixed with 50 µl Phosphate-buffered saline (PBS) inside the microtiter plate and then serial dilutions from 1:2 to 256:1 were prepared from serum. In the next step, 50ul was added to each well from 2% SRBC suspension and then placed at room temperature for 4 to 5 hours. The titers were expressed based on  $\text{Log}_2$  of the highest dilution showing complete agglutination (Pourhossein *et al.* 2015). In order to investigate the Newcastle disease (NDV) and influenza on 28 and 42 days of age, two birds from each pen were taken for blood samples and then the samples were merged and then to evaluate the serum levels of Newcastle disease and influenza, hemagglutination inhibition (HI) test was performed on the samples according to OIE standard (Office international des epizooties). 96-well microplates were used for the experiment. First, 25 microliters of PBS (Peripheral Blood Smear) were poured into all the wells, then 25 microliters of bird serum were poured into the first well and its dilution was performed until the last well. In the next step, 25 microliters of NDV and influenza antigens were added to all of the wells. Then the microplate was put on the mechanical shaker for 1 minute and the microplate was then placed at 25°C for 30 min. In the next step, 25 microliters of 1% red blood cells were added to all the wells, and the microplate was again placed on a mechanical shaker for 15 seconds, and then the microplate was placed at 25°C for 30 minutes and the results were recorded. A 4-unit antigen (Pasouk, Iran) was used to perform the HI test. The titers were diluted based on  $\text{log}_2$ . The red blood cell 1% was also obtained from SPF (Sun Protection Fector) chicks. On the day 42, two birds from each pen were taken for blood samples to count the total number of white blood cells and their differential counts, and their blood was transferred to the tubes containing anticoagulants after fusion. Determining the blood cells was performed by staining, cell differentiating and eye counting with optical microscope (Seidavi *et al.*, 2014). In addition to examining

the effect of aspirin on the immune system, at the end of the experiment after two hours of starvation, 2 birds per replicate with a weight near to the mean were slaughtered. The weights of spleen, bursa of Fabricius and thymus were measured using a digital scale with an accuracy of 0.01g (A&D GF-300 digital scale balance (310 g × 0.001 g, A&D Weighing Design and Manufacture, San Jose, CA)) (Shabani *et al.*, 2015).

### **Microbial flora**

To investigate cecal microflora, two birds of each treatment were slaughtered on day 42 and after opening the abdominal cavity, the right and left cecum were separated with sterile scissors and the contents were discharged into sterile microtubules and were maintained until microbial culture to examine the *Escherichia coli* population at 20°C (Dibaji *et al.*, 2014). To dilute the samples, successive dilution (1 to 10 ratio) in distilled water that had been autoclaved at a pressure of 120atm. One gram of each frozen sample was added after defrosting to 9 ml of distilled water to form a series of dilutions from 10<sup>-1</sup> to 10<sup>-6</sup>. Then 300 µl of each of the dilution series, 10<sup>-3</sup>, 10<sup>-4</sup>, and 10<sup>-5</sup> was taken and inoculated on the plates containing the culture medium and spread completely on the culture surface by the loop. The cultivation was performed next to the flame and under the laboratory hood. The samples inoculated at 37°C for 24 hours for the growth of *Escherichiacolibacteria* in the culture medium EMB (Eosin methylene blue Agar) were placed in incubation (Jang *et al.*, 2007). Then, counting the colonies formed in the most suitable dilution n (10<sup>-4</sup>) was performed to determine the CFU (Colony Forming Units). After the counting, the number of colonies on each culture medium was multiplied in the inverse of dilution. Due to the magnitude of the numbers obtained from the bacterial count, in order to facilitate the calculations, the base-10 logarithm of the numbers was calculated and then used to analyze the data (Dibaji *et al.*, 2014).

### **Profile of fatty acids**

In order to measure the profile of fatty acids in 42 days from each treatment, one bird was slaughtered and the sample of breast meat was transferred to the laboratory. To do this, the muscle breast was first isolated, then ground and kept in a freezer at -20°C (to protect the tissue and compound of the flesh). The fat content of the samples was extracted by the method of Folch *et al.* (1957). First a mixture of two strong solvents of chloroform and methanol was prepared with the ratio of 2 to 1, respectively. The amount of one gram of the mixed samples was then weighed and poured into the closed test tubes. Then 15 cc of the prepared solvent was added to it and thoroughly mixed and then kept in the refrigerator for 24 hours. After the above time, 5 cc of distilled water was added to the samples to create 3 phases in the test tube. After separating the bottom phase, which contains chloroform and dissolved fat, this part was poured into special centrifugal tubes and centrifuged at 25°C with 300 rpm for 15 minutes until the phases of chloroform and fat were completely separated. This process was performed twice, and at the end, after final centrifugation of the bottom phase, which contained only chloroform and dissolved fat, it was separated and poured into a clean laboratory container. After the solution was poured into the test tube, the tube was placed under the laboratory hood in 70°C water and nitrogen gas was blown on it to evaporate the chloroform. Then, 50 mg of pure extracted fat was removed and it was treated with base

and acid methylation in two stages. First, the fat sample was kept for 30 minutes under the influence of 0.5M methoxide solution in methanol at 50°C and then under the influence of chloridric acid solution in methanol (1:1 ratio) for 30 minutes at 50°C. The methyl ester of fatty acids produced in hexane was dissolved by adding hexane to the solution. Then, by adding dry sodium sulfate to the solution and final dehydration, methyl esters of fatty acids dissolved in hexane were passed through a special filter and prepared for injection into the column of chromatographic gas device (Agilent America, 7890B GC Series). C13 fatty acids were used as the internal standard. Pure nitrogen was used as the carrier gas for injection into the chromatographic gas device in a ratio of 1 to 50. The temperature schedule used for the column was such that the temperature of the oven was kept constant for 4 minutes at 100°C. Then, it reached a temperature of 240°C at a rate of 3°C per minute, and was then kept at a constant temperature of 240°C for 20 min. The temperature of injector was 225°C and the detector temperature was 250°C. The analysis time of each sample was 71 minutes and it should be noted that the nitrogen gas pressure inside the column was 2.2, the hydrogen gas pressure was 0.5 and the air pressure was 0.4 kg/m<sup>2</sup>.

### **Statistical Analysis**

All data collected during the experiment and laboratory traits were analyzed by SAS statistical software based on completely randomized design (CRD). The comparison of the means was performed with Duncan's multiple-range test at 5% statistical level.

## **Results And Discussion**

### **Growth performance**

The results of the effects of using different levels of aspirin on the performance of broilers are given in Tables 2 and 3. The results showed that although weight gain in the period 1 to 10 days at two levels of aspirin did not show a statistically significant difference, weight gain in birds in the two aspirin treatments was significantly greater compared to the control group ( $P < 0.05$ ) and the highest weight gain was related to A50 level. FCR (Feed conversion ratio) for the period 1 to 10 days was significant ( $P < 0.05$ ) greater but did not show a statistically significant difference compared to the control group. In addition, the best FCR was for the 50A level. Feed intake and weight gain of 11 to 24, 25 to 42 and 1 to 42 days statistically showed no significant difference between the two levels of aspirin, but they were significantly improved compared to the control group ( $P < 0.05$ ); the highest feed intake and the highest weight gain was for A100 except for the period 11 to 24 days. In addition, the best FCR of during the 25 to 42 days period was for the same level of A ie (A100). However, FCR of 1 to 42 days of age did not show any significant difference between the two levels of A, but it was significant compared to the control group; and the best FCR was related to A100.

According to the results, although the liveweight of aspirin-treated chicks at the 42nd day of age (g/chick), feed cost per kg live weight (Rial/kg) and the European factor were not significant between the two different levels of A, but they both showed a statistically significant difference compared to the control

group ( $P < 0.05$ ). The lowest cost of liveweight of chicken and the best European factor was related to A 100. In fact, aspirin, like some other vitamins, acts as an antioxidant, and by reducing the production of prostaglandins and also by lowering blood viscosity caused by an increase in blood pH, has a role in reducing the production of free radicals. Increasing alkalinity in the blood can lead to high blood pressure, so aspirin can have a role in maintaining the structure of the heart and dilate blood vessels thereby improving blood flow to important organs such as the liver and kidneys, resulting in an improved performance (Zhang *et al.*, 2016). Abdel-Fattah (2006) and El-Soud *et al.* (2006) also reported that using aspirin supplement in the diet of broilers results in a better production performance. Similar results are reported in the findings of Jebur *et al.* (2018) and Rokade *et al.* (2017) (again these were higher doses of aspirin). In fact, aspirin by inhibiting the formation of free radicals and protecting against oxidative damage in tissues and liver cells improved the performance of broilers (Jebur *et al.*, 2018).

### **Carcass characteristics, carcass fat and digestive organs**

The effect of experimental treatments on carcass characteristics is shown in Tables 4 and 5. The results showed that the two levels of aspirin were not significantly different in live weight, featherless weight, full body weight, empty body weight, eviscerated carcass percentage, breast percentage, thigh percentage and ventricular fat, but both aspirin treatments were significantly improved compared with the control group ( $P < 0.05$ ). According to the table, the lowest ventricular fat was related to the two levels of aspirin which is consistent with the results of Rokade *et al.* (2017), in which it was reported that aspirin consumption could significantly reduce corticosterone and serum cholesterol and ventricular fat. Two different levels of aspirin did not significantly affect the relative weight of the pancreas ( $P \geq 0.05$ ). In addition, the two different levels of A did not have any significant effect on the relative weight of gizzard, heart, and pre-stomach, but there was a statistically significant difference compared to the control group ( $P < 0.05$ ) which is also consistent with the findings of Rokade *et al.* (2017). Aspirin may have improved the performance of broiler chickens by inhibiting the formation of free radicals and protecting against free radical damage in liver tissues and cells (Jebur *et al.*, 2018).

### **Parts of intestine**

The effect of experimental treatments on different parts of the intestine is shown in Table 6. The results showed that the use of two different levels of aspirin on the weight ratio of jejunum, ileum, colon and right cecum were not statistically significant different from each other, but both aspirin treatments were significant compared to the control group ( $P < 0.05$ ) and the highest weight ratio was related to the level A100. According to the findings of Jebur *et al.* (2017), the use of vitamins C, E, aspirin, and sodium chloride in the diet of broilers led to positive effects on digestibility, weight ratio, and some carcass characteristics, which was consistent with the findings of Stilborn *et al.* (1988). These results could be related to the antioxidant properties of this compound, which by removing free radicals caused by breeding conditions can lead to improving the relative weight and some carcass characteristics. The use of aspirin had no significant effect on the relative weight of the rectum, duodenum and left cecum ( $P \geq 0.05$ ).

## Blood parameters and digestive enzymes

The results of using two different levels of aspirin on blood parameters and liver enzymes are shown in Tables 7 and 8. The results showed that the effects of two different levels of aspirin on blood parameters and liver enzymes of broilers except from alkaline phosphatase were not significant throughout the period ( $P \geq 0.05$ ). These results were also observed by Abdulameer (2019). The use of two different levels of aspirin on alkaline phosphatase was not significant. The lowest activity of this enzyme was at A50, but it was significant compared to the control group ( $P < 0.05$ ). Compounds that have antioxidant properties can affect blood parameters (Sahin *et al.*, 2002). Recently, medical experiments have shown that antioxidants prevent the reduction of cholesterol in the body, which is also consistent with the results of this study. Naturally, the liver cells break down key proteins in the structure of lipoproteins, such as VLDL. This means that VLDL will not be converted to LDL which is the most important cholesterol carrier in the blood. In fact, antioxidants prevent this from happening in the liver cells. So, no oxidation of apoprotein B in VLDL means the continuation of the metabolism of lipoproteins and the formation of LDL (Krauss., 2004). Indeed, according to the results, using the level A100 numerically led to a decrease in HDL and total protein. In addition, the use of the same level of aspirin caused the reduction of glucose concentration.

## Immune system

The effects of adding different levels of aspirin in the diet on the function of the humoral immune system in response to the injection of SRBC antigen and antibody titer against NVD disease and influenza virus are shown in Tables 9 and 10. The results of the use of two levels of aspirin showed that there was no significant effect on the function of the humoral immune system in response to injection of SRBC antigen and antibody titer against Newcastle disease and influenza virus ( $P \geq 0.05$ ). However, the highest percentage of neutrophils and eosinophils were observed in the diets containing 100 mg/kg aspirin, and the lowest percentage of lymphocytes was related to the same level of aspirin. In addition, aspirin levels resulted in a significant correlation between relative weight of thymus and bursa of Fabricius ( $P < 0.05$ ) but did not significantly affect spleen weight ( $P \geq 0.05$ ).

## Microbial flora

The effect of aspirin on the population of *Escherichia coli* bacteria is shown in Table 11. The results showed that the population of *Escherichia coli* bacteria using the level A100 decreased compared to the other treatments and the highest population of *Escherichia coli* bacteria was observed in the intestines of the chickens fed by A0. In fact, increases in free radicals can lead to oxidation and destruction of biological cells, so elevated free radical concentrations can cause a number of disorders in intestinal tissue (Ocak *et al.*, 2008; Sahin *et al.*, 2003). Besides, antioxidants, including aspirin, with their ability to effectively inhibit free radical production, may ameliorate the problems related to the intestinal disorders caused by high-temperature and improve functional traits (Wang *et al.*, 2008).

## Profile of fatty acids in breast meat

The effect of the treatments on the percentage of fatty acids in the breast muscle tissue is shown in Table 12. The results showed that the levels of saturated fatty acids such as myristic acid, palmitic acid and stearic acid decreased with increasing aspirin levels. The results also showed a positive effect of high levels of aspirin on the percentage of unsaturated fatty acids such as palmitoleic acid and oleic acid, and the highest increase was related to A100. This is because the storage of n-3 polyunsaturated fatty acids in the breast muscle is greater than in the thigh muscle. Cis-11,14-eicosadienoic acid, cis-8,11,14-eicosatrienoic acid and cis-11,14,17-eicosatrienoic acid are as a standard ideal for biological studies. They are mainly found in small amounts in animal tissues (Huang *et al.*, 2011; Wang *et al.*, 2012). According to the results of the above study, high levels of aspirin resulted in the increase of the levels of cis-8,11,14-eicosatrienoic acid and cis-11,14,17-eicosatrienoic acid. The lowest cis-11,14-eicosadienoic acid was related to the same level of aspirin mentioned above. Cis-11,14-eicosatrienoic acid is produced by an enzyme delta-9 elongase from linoleic acid and can be converted to dihomo- $\gamma$ -linolenic acid, arachidonic acid, sciadonic acid and other unsaturated fatty acids. Cis-11,14-eicosadienoic acid is able to adjust unsaturated fatty acids and is responsible for the response of macrophages to inflammatory stimulus. Along with other monounsaturated fatty acids, cis-11,14-eicosadienoic acid can inhibit the binding of leukotriene B<sub>4</sub> to the neutrophil membrane, which is part of these anti-inflammatory activities (Huang *et al.* 2011). According to Ghalib *et al.* (2011), aspirin also has this antioxidant activity; which means that aspirin can protect the cell membrane and the unsaturated fatty acids of the membrane against the oxidation of free radicals.

## Conclusion

In general, it could be stated that according to the present study, the use of aspirin in the diet of broilers of Ross 308 strain improved feed intake, weight gain, conversion ratio, cost per kilogram of live weight and production factor. Although aspirin did not influence the blood parameters measured here, it improved the immune system and reduced ventricular fat, resulting in improved carcass meat quality. Also, the use of 100 mg/kg levels of aspirin led to an increase in unsaturated fatty acids and a decrease in saturated fatty acids in meat, which is beneficial to human health. Therefore, according to the results of this experiment, the use 100 mg/kg aspirin is recommended in the diets for growing broiler chickens as an antioxidant compound and a cheap growth stimulant.

## Declarations

### Conflicts of interest

The authors declare no conflicts of interest in this work.

### Ethics approval

All procedures involving animals were in compliance with the European Community Council Directive of 24 November 1986, and ethical approval was granted by the Rasht branch, Islamic Azad University Ethics

Committee (No. 14 10 2018, Rasht, Iran.

### **Authors' contribution**

MT, MB and AS: conceptualization, original manuscript writing, editing; MT: investigation, data collection; MB and AS: resources, review and editing

### **Financial support**

Financial supported by Rasht Branch, Islamic Azad University: Grant number 17.16.4.18418 is gratefully acknowledged.

### **Consent to participate**

Not applicable.

### **Consent for publication**

All data and materials are ready to publication.

### **Code availability**

Not applicable

## **References**

1. **Abdel, F. S.** (2006). Physiological and immunological adjustments of dietary ascorbic acid and acetyl salicylic acid in heat stressed Japanese quail. *Egyptian Poultry Science*. 26(4): 1395-1418.
2. **Abdulameer, Y. S.** (2019). The effects of dietary vitamin C and *Citrus sinensis* peel on growth, hematological characteristics, immune competence, and carcass characteristics in broilers exposed to heat stress. *Iraqi Journal of Veterinary Sciences*, 32(2), 253-260.
3. **Akbari, M., Kermanshahi H., & Keydari G.**(2004).Investigation of the addition of acetic acid in drinking water on yield, growth indices and microbial population of Ileum broilers. *Journal of Agricultural Sciences and Technology and Natural Resources*, 3: 139-146
4. **Alagawany, M., Farag, M. R., El-Hack, M. A., Dhama, K., & Fowler, J.** (2017). Use of acetylsalicylic acid as a feed additive in poultry nutrition. *World's Poultry Science Journal*, 73(3), 633-642.
5. **Al-Obaidi, F. A., & Al-Shadeedi, S. M.** (2010). Effect of dietary aspirin for reducing ascities and enhancing productive performance of broilers reared in high density. *Al-Qadisiya Journal of Veterinary Medicine Science*, 9, 20-25.
6. **Dibaji, S. M., Seidavi, A., Asadpour, L., & Moreira da Silva, F.** (2014). Effect of a synbiotic on the intestinal microflora of chickens. *Journal of Applied Poultry Research*, 23(1), 1-6.

7. **El-Soud, S. B. A., Ebeid, T. A., & Eid, Y. Z.** (2006). Physiological and antioxidative effects of dietary acetyl salicylic acid in laying Japanese quail (*Coturnix japonica*) under high ambient temperature. *The Journal of Poultry Science*, 43(3), 255-265.
8. **Folch, J., Lees, M., & Stanley, G. S.** (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of biological chemistry*, 226(1), 497-509.
9. **Ghalib ,Rawaa , AthraaFalah, Adel Ahmad .**(2011). Ulcerogenic effects of NSAIDs on gastric mucosa: comparison between selective and non-selective COX2 inhibitors; indomethacin VS rofecoxib. *Medical Journal of Babylon -vol.8, NO.2.*
10. **Golrokh, A. J., Bouyeh, M., Seidavi, A., van den Hoven, R., Laudadio, V., & Tufarelli, V.** (2016). Effect of different dietary levels of atorvastatin and L-carnitine on performance, carcass characteristics and plasma constituents of broiler chickens. *The Journal of Poultry Science*, 53(3), 201-207.
11. **Gore, A. B., & Qureshi, M. A.** (1997). Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. *Poultry Science*, 76(7), 984-991.
12. **Huang, Y. S., Huang, W. C., Li, C. W., & Chuang, L. T.** (2011). Eicosadienoic acid differentially modulates production of pro-inflammatory modulators in murine macrophages. *Molecular and cellular biochemistry*, 358(1-2), 85-94.
13. **Jang, I. S., Ko, Y. H., Kang, S. Y., & Lee, C. Y.** (2007). Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Animal Feed Science and Technology*, 134(3-4), 304-315.
14. **Jebur, S. F., Hussain, F. M. H., & Mohammed, T. T.** (2017). The effect of adding different levels of Vitamin C, E, Aspirin and Sodium Chlorides in diet for total digestible nutrients, and some organ relative weights and carcass traits of broiler chickens during heat stress. *Al-Anbar Journal of Veterinary Sciences*, 10(1), 94-107.
15. **Jebur, S., MOHAMMED, T., & Firas, A. K.** (2018). Effect of Vitamin E, C and Aspirin in the Performance, Lipid Peroxidation and Blood Biochemistry Traits of Broiler in Heat Stress. *The Eurasia Proceedings of Science Technology Engineering and Mathematics*, (3), 145-151.
16. **Krauss, R. M.** (2004). Hold the antioxidants and improve plasma lipids? *The Journal of Clinical Investigation*, 113(9), 1253-1255.
17. **Lerner, K. G., Glick, B., & McDuffie, F. C.** (1971). Role of the bursa of Fabricius in IgG and IgM production in the chicken: evidence for the role of a non-bursal site in the development of humoral immunity. *The Journal of Immunology*, 107(2), 493-503.
18. **Madamanchi, N. R., Vendrov, A., & Runge, M. S.** (2005). Oxidative stress and vascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25(1), 29-38.
19. **Manual, R. B.** (2012). Ross 308 broiler nutrition manual. *Ross Broiler Ltd: Newbridge, Midlothian, UK.*
20. **Nowak, J. Z.** (2014). Aspirin and age-related macular degeneration: positives versus negatives. *Expert Opinion on Drug Safety*, 13(6), 687-690.
21. **Ocak, N., Erener, G., Burak Ak, F., Sungu, M., Altop, A., & Ozmen, A.** (2008). Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.) leaves

- as growth promoter source. *Czech Journal of Animal Science*, 53(4), 169.
22. **Pourhossein, Z., Qotbi, A. A. A., Seidavi, A., Laudadio, V., Centoducati, G., & Tufarelli, V.** (2015). Effect of different levels of dietary sweet orange (*Citrus sinensis*) peel extract on humoral immune system responses in broiler chickens. *Animal Science Journal*, 86(1), 105-110.
  23. **Poźniak, B., Yuan, H., Yi, J., Guo, C., Deng, S., Zhu, L., ... & Świtajła, M.** (2010). Influence of salicylate administration on weight gain and serum biochemical parameters in broiler chickens. *Medycyna Weterynaryjna*, 66 (10), 675-679.
  24. **Richard, L.M.** (2007). The 100 most important chemical compounds: a reference guide. ABC-CLIO. p. 10. ISBN 978-0-313-33758-1.
  25. **Rokade, J. J., Bhanja, S. K., Shinde, A. S., Bhaire, D. B., & Mandal, A. B.** (2017). Evaluation of aspirin (ASA) in broiler chicken during hot dry summer using zoo technical, molecular and physio-biochemical tools. *Indian Journal of Animal Research*, 51(1), 97-104.
  26. **Sahin, K., Orhan, C., Smith, M. O., & Sahin, N.** (2013). Molecular targets of dietary phytochemicals for the alleviation of heat stress in poultry. *World's Poultry Science Journal*, 69 (1), 113-124.
  27. **Sahin, K., Sahin, N., & Yaralioglu, S.** (2002). Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites, and mineral concentrations of laying hens reared at high ambient temperature. *Biological Trace Element Research*, 85(1), 35-45.
  28. **Sahin, K., Sahin, N., Onderci, M., Gursu, M. F., & Issi, M.** (2003). Vitamin C and E can alleviate negative effects of heat stress in Japanese quails. *Food, Agriculture and Environment*, 1(2), 244-49.
  29. **Seidavi, A., Asadpour, L., Dadashbeiki, M., & Payan-Carreira, R.** (2014). Effects of dietary fish oil and green tea powder supplementation on broiler chickens' immunity. *Acta Scientiae Veterinariae*, 42(1), 1-13.
  30. **Shabani, S., Seidavi, A., Asadpour, L., & Corazzin, M.** (2015). Effects of physical form of diet and intensity and duration of feed restriction on the growth performance, blood variables, microbial flora, immunity, and carcass and organ characteristics of broiler chickens. *Livestock Science*, 180, 150-157.
  31. **Sigolo, S., Deldar, E., Seidavi, A., Bouyeh, M., Gallo, A., & Prandini, A.** (2019). Effects of dietary surpluses of methionine and lysine on growth performance, blood serum parameters, immune responses, and carcass traits of broilers. *Journal of Applied Animal Research*, 47(1), 146-153.
  32. **Stilborn, H. L., Harris Jr, G. C., Bottje, W. G., & Waldroup, P. W.** (1988). Ascorbic acid and acetylsalicylic acid (aspirin) in the diet of broilers maintained under heat stress conditions. *Poultry Science*, 67(8), 1183-1187.
  33. **Truong, A., Sayago, M. M., Kutteh, W. H., & Ke, R. W.** (2016). Subchorionic hematomas are increased in early pregnancy in women taking low-dose aspirin. *Fertility and Sterility*, 105(5), 1241-1246.
  34. **Valko, M. M. H. C. M., Morris, H., & Cronin, M. T. D.** (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, 12(10), 1161-1208.
  35. **Wang, L., Piao, X. L., Kim, S. W., Piao, X. S., Shen, Y. B., & Lee, H. S.** (2008). Effects of *Forsythia suspensa* extract on growth performance, nutrient digestibility, and antioxidant activities in broiler

chickens under high ambient temperature. *Poultry Science*, 87(7), 1287-1294.

36. **Wang, X., Lin, H., & Gu, Y.** (2012). Multiple roles of dihomo- $\gamma$ -linolenic acid against proliferation diseases. *Lipids in Health and Disease*, 11(1), 25.
37. **Whiting, T. L., Drain, M. E., & Rasali, D. P.** (2007). Warm weather transport of broiler chickens in Manitoba. II. Truck management factors associated with death loss in transit to slaughter. *The Canadian Veterinary Journal*, 48(2), 148.
38. **Wu, D., Zhang, M., Lu, Y., Tang, S., Kemper, N., Hartung, J., & Bao, E.** (2016). Aspirin-induced heat stress resistance in chicken myocardial cells can be suppressed by BAPTA-AM in vitro. *Cell Stress and Chaperones*, 21(5), 817-827.
39. **Zhang, X. H., Zhu, H. S., Qian, Z., Tang, S., Wu, D., Kemper, N., ... & Bao, E. D.** (2016). The association of Hsp90 expression induced by aspirin with anti-stress damage in chicken myocardial cells. *Journal of Veterinary Science*, 17(1), 35-44.

## Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables.docx](#)