

Original Research Article

The effect of adding Mintmint and lemon to heat-stress Broiler's drinking water

Abstract

Heat stress adversely affects livestock species. In chickens, heat exposure disrupts metabolic and immune system functions and increases mortality. Therefore, investigating these factors will improve animal adaptability and productivity. Several studies showed that using feed additives modified the physiological functions and responses of heat-stress birds. Our goal is to investigate whether mint and lemon ingredients can improve broiler performance characteristics under heat-stress conditions. A total of 120 - one-day-old Hybrid chicks were assigned to four experimental groups, each with three replicate pens of 30 chicks. The watering treatments included the control group, and water with Mintmint, water with lemon, and mint-lemon groups, respectively. The study's results showed that the incorporation of Mint-mint and lemon significantly enhanced the growth performance of these chickens. Physiological (body weight, weight gains, feed intake, carcass weight, and internal organs) and behavioural measurements showed better mint-lemon mixed chickens' performance than the control group under the same rearing system. The study concluded that Mint-mint and lemon could be effectively used in broiler feeding to reduce the impact of high environmental temperatures.

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Key Words: Broiler, Heat stress, Mint, Lemon, Physiology, Behaviour

1. Introduction

Climate change will majorly impact Middle Eastern countries, with temperatures expected to increase by 1-2 °C by 2030-2050 (IPCC, 2013). Soil moisture is projected to decrease, leading to increased soil degradation and vegetation-associated impacts. Water resources, grasslands, and livestock are thus likely to be vulnerable to climate change in this region. Climate change affects animal health mainly through heat stress and increases in vector-borne diseases. Animals suffering from heat stress have been the topic of intensive studies over the last 50 years (reviewed in Collier *et al.*, 2017). These studies have shed light on how normal physiological processes are altered by stress and how these processes, in turn, affect critical biological functions such as reproduction, growth, and immunity.

Nevertheless, our understanding of the mechanisms underlying animals' physiological and behavioural responses to heat stress is in its infancy. Significant advances are needed to enable the livestock industries to adapt to climate change, minimizing impacts on production and welfare. Consequently, there is a growing need to develop and validate reliable behavioural and physiological indicators for heat stress. Promising molecular indicators include hormonal regulators of metabolism and body temperature (e.g., the thyroid hormone triiodothyronine (T3), which controls many physiological processes

including body temperature; its prohormone and metabolic stimulator thyroxine (T4); aldosterone which influences water retention and loss; prolactin which regulates immune function, metabolism and lactation; and growth hormone), and acute-phase proteins (APP), which are markers of inflammation (e.g., Najafi *et al.*, 2015). Immune function is compromised by various stressors, including poor nutrition, extreme temperatures, injury, and disease. The major problem in the Middle East and Gulf areas is a shortage of feed resources and prolonged drought that negatively affect animal productivity and sustainability. Therefore, investigating these factors will improve animal adaptability and productivity, improving the agriculture sector in these countries.

In modern broiler farm practices, alternative methods for using safe substances are employed to comply with consumer demands for healthy food. In the traditions of many cultures, Mint (*Mentha longifolia*) has been considered a healing herb and food since antiquity. Mint extracts contain hydrocarbons, thymol, and highly oxygenated compounds, which are believed to be responsible for their beneficial characteristics (Elansary and Ashmawy, 2013). The composition of essential oils from Mint-mint exhibits strong antibacterial (Elansary and Ashmawy, 2013) and antioxidant activities (Elansary and Ashmawy, 2013). The stems and leaves of Mint-mint have several therapeutic properties (Mikaili *et al.*, 2013). The leaves contain essential oils that are beneficial for digestion. According to Ocak and his colleagues (2008), Mint-mint positively affects broilers' growth. Additionally, previous studies have shown that mints keep poultry guts healthy and well-balanced with normal microflora (Asadi *et al.*, 2017). Consequently, the qualities of chilled and frozen broiler meat are improved by supplementation with Mint-mint during the rearing

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process. Several research studies showed that vitamin C administration could enhance chicken growth and metabolism by neutralizing oxygen radicals and reducing oxidative damage (Hieu *et al.*, 2022; El-Senousey *et al.*, 2018). Vit C is also a potent scavenger of free radicals in biological systems (Hieu *et al.*, 2022; El-Senousey *et al.*, 2018). It assists the body in defending against free radical damage to proteins and lipid membranes and has antioxidant properties; it is an excellent poultry feed additive. Likewise, Vit C is an essential reducing agent in biological systems and a free radical scavenger under heat-stress conditions. By preventing free radical damage and protecting proteins and lipid membranes from oxidative stress, Vit C enhances birds' immune systems and antioxidant capacity (Hieu *et al.*, 2022). Under heat-stress conditions, broiler chickens were found to have improved immunity when lemon juice was added to their drinking water (Kadam *et al.*, 2009). It has been demonstrated that Vit C contributes to poultry birds' energy supply by facilitating corticosterone biosynthesis in a heat-stress environment (Abidin and khatoun, 2013). Vit C has been shown to modify physiological functions and treat and prevent Salmonella (Mousavi *et al.*, 2019). Hence, combining Mint-mint and Vit C could improve immunity and performance in broilers because their active components have synergistic and suppressive effects. Our goal is to investigate whether mint (*Mentha longifolia*) and lemon (Vitamin C) ingredients can improve broiler chicken production and some performance characteristics (Live and carcass weights, feed, and water intake) in the broiler production cycle.

2. Material and Methods

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Experimental animals were used according to all local laws, guidelines, and policies at Jerash University - Jordan.

This investigation used one hundred and twenty-one-day-old mix-sex chicks (Hybrid). Birds were caged in the same experimental room using wire cages. A commercial vegetable-based feed ration (according to NRC requirements) and water were provided *ad libitum* during the experiment (42 days). The chicks were fed a starter (CP: 22%; ME: 3014 kcal) during the first two weeks, a grower (CP: 20 %; ME: 3058 kcal) during weeks 3-4, and a finisher diet (CP: 18%; ME: 3115 kcal) during weeks 5-6. Birds were managed routinely, with the average air temperature and relative humidity being 28.1 ± 0.5 °C and 15.0 ± 0.4 , respectively. A period of 23 h of light was followed by one h of darkness in the lighting program. Thirty chicks, each with three replicate pens of ten chicks, were weighed and randomly allocated to the four groups. The water treatments included tap water (control), water with mint extraction (0.3%), water with lemon extraction (0.3%), and water with the mint-lemon combination (0.15% each) groups, respectively. The Mint was soaked in warm water (Threelitres), covered, and left for 60 minutes, drained, added to sixlitres of water, and served to birds. During the squeezing of lemons, the liquid was collected, and the remaining peel and pulp were air dried, then ground and added to warm water (Threelitres) and covered well for 60 minutes. Then, the solution was drained, added to sixlitres of water, and served to the chickens.

Chickens were weighed weekly. Feed consumption per cage for seven days was determined during the experimental period. Hens were inspected daily, and mortality was recorded with the cause of death if known. Carcass yield and internal organs were measured at the end of

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the experiment: Feed intake, body weight, and growth rate were calculated during the investigation.

Behavioural data were collected using live observations. Birds performing drinking and feeding activities were recorded by observers in each cage every five minutes during observation hours, allowing for tabulating the number of chickens eating and drinking three times a day at 0800, 1200, and 1500 h.

2.1 Statistical Analysis

This study used a randomized design and an ANOVA involving repeated measures (MIXED model) procedures for analyzing collected data (SAS Institute, 2001). As dependent variables, the performance traits of the birds were included in the model. The independent fixed effects consist of the experiment's impact (control or water supplementation), the age in a week, and the respective interaction. The bird was included as a random effect. Tukey-Kramer test separated the mean differences among different treatments. A significant level was considered when $P < 0.05$. All values were expressed as $LS_{\text{means}} \pm SE$ unless otherwise mentioned.

3. Results and Discussion

Heat stress interferes with birds' ability to synthesize ascorbic acid. Therefore, the effects of Mint-mint and lemon addition to drinking water, either sole or mixed, under heat-stress conditions are shown in Tables 2 and 3. In the first two weeks, neither the Mint-mint nor Lemon-lemon affects significantly on the birds' performance ($P > 0.05$), whereas the mixed combination of Mint-mint and Lemon-lemon improved feed intake, body weight, and feed conversion ratio ($P < 0.05$) starting from the third week of age until the end of the study. When using the mixture of Mint and lemon, eating and weight gains improved significantly

compared to using each separately ($P < 0.05$). Carcass and eviscerated yields increased in weight as the body weight increased, as shown in Table 4, with a heavier carcass weight when we offered combined Mint and Lemon or lemon showed higher carcass and thigh weight compared to the control and Mint group ($p < 0.05$). Furthermore, water treatments showed significantly higher chest weight and lower abdominal fat weight compared to the control group ($P < 0.05$). No significant differences in back, wing, neck, and liver weight were found in treated and untreated groups ($P > 0.05$).

Behavioural observation demonstrated that the control group visited feed (3.50 N/day) and water (3.26 N/day) troughs several times lower than the treatment groups (Figure 1). Mint and lemon mixed combination treatment showed higher drinking (3.87 N/day) and feeding (4.20 N/day) activities compared to the mint (3.42 and 3.81 N/day) and lemon (3.40 and 3.91 N/day) groups for drinking and feeding respectively.

Heat stress is a significant stressor resulting in decreased bird welfare and meat quality. Due to heavy feathers, poultry cannot sweat and cannot release heat into their environment through their body feathers. Therefore, broilers in hot environments perform several physiological and behavioural mechanisms to cope with the hot climate. Heat stress birds reduce their feed consumption and activity, increase water intake and body temperature, rest during the heat stress, and spread their wings and pant to promote cooling by reducing body insulation (Bohler *et al.*, 2021). Reduced feed intake is presumed to contribute to the most detrimental effects of heat-stressed birds' productivity (Collier *et al.*, 2017). Abidin and Khatoon (2013) showed that birds' plasma had a lower antioxidant vitamin C concentration, thus, increasing the oxidative damage of heat-stressed birds; additionally, the

acid-base balance of birds is disturbed by the panting process. Therefore, it is essential to inhibit stress production to reduce the adverse effects of elevated temperatures.

Therefore, it is essential to inhibit stress production to reduce the adverse effects of heat stress. Parallel to our results, Spirling and Daniels (2001) found that Mint positively affects the digestive process and influences feed intake due to its pharmacological properties, through enhancing intestinal function, bile production, microbial growth, bacteria formation, erratic activity, and choleric activity (Kim *et al.*, 2022; Mimica-Dukic *et al.*, 1999). Additionally, adding lemon juice (Vit C) to drinking water enhanced the immunity of broiler chickens and protected against reactive oxygen species by preventing free radicals from forming at high temperatures (Kadam *et al.*, 2009). It has been shown that adding lemon to poultry diets increases body weight, reduces mortality, and improves performance, feed intake, and feed efficiency (Abidin and khatoun, 2013). Furthermore, it improves birds' immune response and antioxidant capacity, stimulating the synthesis of corticosterone to support the energy supply of poultry at high temperatures (Kadam *et al.*, 2009).

Therefore, adding a mixture of Mint-mint and lemon can enhance broiler performance and immunity; their complementary, repressing actions and active ingredients improve feed efficiency and digestive function by decreasing the effects on gastrointestinal disorders. As well as inhibiting harmful bacteria from growing in the digestive system, Mint's antiseptic properties help digestion and absorption; at the same time, Vit C improves immunological status. Thus, combining Mint-mint and lemon juice has successfully enhanced broilers' feed intake, resulting in improved performance. Drinking and feeding behaviour showed that once the feed is consumed, the birds spend their time drinking, which reflects the higher

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feeding and drinking behaviour of chickens that receive a combination of Mint-mint and lemon in their water.

Fat deposition is considered a negative feature since it represents a nutritional loss; it may also contribute to decreased carcass yields and meat consumption (Fouad and El-Senousey, 2014). Therefore using Mint and lemon juice might reduce hepatic lipogenesis and stimulate fatty acid metabolism by promoting fatty acid breakdown via enhanced digestion due to beneficial digestion and choleric effects on fat deposition inside the body compared with the control group.

3. Conclusion

In conclusion, using Mint-mint and lemon as their active components synergize effectively alleviates the harmful impacts of high temperatures on broiler chickens. Consequently, adding Mint-mint and lemon to poultry diets can improve body weight and reduce carcass fatness. Further studies should focus more on applying Mint-mint and Lemon-lemon as an alternative to antibiotics.

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Table 1: Nutritional composition of starter, grower, and finisher diets

Diet	Starter	Grower	Finisher
	1-2 wk	3-4 wk	5-6 wk
Ingredients and composition			
Yellow corn	61.9	68.5	73.3
Soybean meal (44% CP)	35.5	28.8	24
Dicalcium-phosphate	2.0	2.0	2.0
Premix*	0.12	0.2	0.2
DL-methionine	0.1	0.1	0.1
Choline	0.1	0.1	0.1
Salt	0.3	0.3	0.3
Nutrient chemical composition **			
ME (kcal kg ⁻¹)	3014	3058	3115
Crude Protein	22.2	19.9	18.1
ME/CP	135.7	153.6	172
Calcium (%)	1.07	0.95	0.94

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Phosphorus (%)	0.76	0.74	0.73
Lysine (%)	1.26	1.09	1.02
Methionine (%)	0.49	0.48	0.46
Methionine and cystine (%)	0.89	0.75	0.69
Sustain (%)	0.40	0.39	0.34

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*: One kilogram of premix consists of 12,000,000 IU Vit. A, 2,500,000 IU Vit. D3, 1 g Vit. E, 2 g Vit. K3, 1 g Vit. B1, 5 g Vit. B2, 0.01 g Vit. B12, 30 g Nicotinic acid, 3 g Ca-pantothenate, 1 g folic acid, 0.05 g biotin, 40 g Fe, 5 g CU, 60 g Mn, 0.1 g I, 60 g Zn, 0.15 g Co, 10 g BHT

** : According to NRC tables (1994), the chemical compositions of feed ingredients were calculated.

Table 2: The average body weight of birds when supplementary Mint, lemon, and a combination of Mint and lemon extraction are added to drinking water (0.3%)*

Age (wk)	Control	Mint (0.3%)	Lemon (0.3%)	Mint&Lemon
First	157.93 ± 5.07 ^{Ac}	161.88 ± 5.07 ^{Ac}	160.05 ± 5.07 ^{Ac}	162.48 ± 5.07 ^{Ac}
Second	364.97 ± 5.07 ^{Ac}	365.87 ± 5.07 ^{Ac}	365.22 ± 5.07 ^{Ac}	367.80 ± 5.07 ^{Ac}
Third	672.67 ± 5.07 ^B	681.83 ± 5.07 ^{Bd}	683.27 ± 5.07 ^{Bd}	711.63 ± 5.07 ^{Ac}
Fourth	1158.97 ± 5.07 ^B	1160.67 ± 5.07 ^{Bc}	1183.27 ± 5.07 ^{Bc}	1206.20 ± 5.07 ^{Ac}
Fifth	1505.10 ± 5.07 ^B	1527.37 ± 5.07 ^{Be}	1569.43 ± 5.07 ^{Ad}	1622.87 ± 5.07 ^{Ac}
Sixth	2057.10 ± 5.07 ^B	2117.97 ± 5.07 ^{Ae}	2216.80 ± 5.07 ^{Ad}	2277.77 ± 5.07 ^{Ac}

* A row of values with different superscripts differs significantly at P<0.05

* A, B: Treatment differences (Control vs Treatment addition)

* c,d,e: Differences within the treatments (Mint vs Lemon vs Combination)

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Table 3: The average feed intake when supplementary Mint, lemon, and a combination of Mint and lemon extraction are added to drinking water (0.3%)*

Age (wk)	Control	Mint (0.3%)	Lemon (0.3%)	Mint&Lemon	Comment [A13]: Write the period length in days.
First	137.60±2.49 ^{Ac}	137.33±2.49 ^{Ac}	137.33±2.49 ^{Ac}	147.33±2.49 ^{Ac}	
Second	249.57±2.49 ^B	264.33±2.49 ^{Ad}	260.33±2.49 ^{Bd}	282.00±2.49 ^{Ac}	
Third	524.93±2.49 ^B	542.00±2.49 ^{Ac}	526.00±2.49 ^{Bd}	547.67±2.49 ^{Ac}	
Fourth	808.33±2.49 ^B	806.00±2.49 ^{Ad}	802.00±2.49 ^{Ae}	835.00±2.49 ^{Ac}	
Fifth	823.67±2.49 ^B	868.00±2.49 ^{Ad}	853.33±2.49 ^{Ae}	924.67±2.49 ^{Ac}	
Sixth	1091.33±2.49 ^B	1221.33±2.49 ^{Ad}	1131.67±2.49 ^{Ae}	1276.00±2.49 ^{Ac}	

* A row of values with different superscripts differs significantly at P<0.05

* ^{A, B}: Treatment differences (Control vs Treatment addition)

* ^{c, d, e}: Differences within the treatments (Mint vs Lemon vs Combination)

Table 4: Average live carcass weight (g/bird) and internal viscera (% of live weight) when supplementary Mint, Lemon, and a combination of Mint and Lemon extraction were added to drinking water (0.3%)*

Variable (g)	Control	Mint (0.3%)	Lemon (0.3%)	Mint&Lemon
Live weight	2078.17±23.83 ^B	2144.17±23.83 ^{Bd}	2189.67±23.83 ^{Ac}	2253.87±23.83 ^{Ac}
Carcass weight	1521.30±20.37 ^B	1592.84±20.37 ^{Bd}	1632.94±20.37 ^{Ac}	1695.11±20.37 ^{Ac}
Chest weight	32.48±0.25 ^B	35.72±0.25 ^{Ac}	35.85±0.25 ^{Ac}	36.67±0.25 ^{Ac}
Thigh weight	29.63±0.31 ^B	31.78±0.31 ^{Bcd}	31.95±0.31 ^{Ac}	32.43±0.31 ^A
Back weight	13.50±0.20 ^A	13.77±0.20 ^{Ac}	13.18±0.20 ^{Ac}	13.02±0.20 ^{Ac}
Wings weight	10.86±0.30 ^A	11.48±0.30 ^{Ac}	11.27±0.30 ^{Ac}	11.53±0.30 ^{Ac}
Neck weight	5.73±0.16 ^A	5.96±0.16 ^{Ac}	6.12±0.16 ^{Ac}	6.05±0.16 ^{Ac}
Abdominal fat weight	6.16±0.53 ^A	2.59±0.53 ^{Bc}	2.55±0.53 ^{Bc}	2.18±0.53 ^{Bc}
Liver weight	3.23±0.09 ^A	3.49±0.09 ^{Ac}	2.85±0.09 ^{Ad}	3.38±0.09 ^{Ae}

* Values within a row with different superscripts differ significantly at $P < 0.05$

* A, B: Treatment differences (Control vs Treatment addition)

* c,d,e: Differences within the treatments (Mint vs Lemon vs Combination)

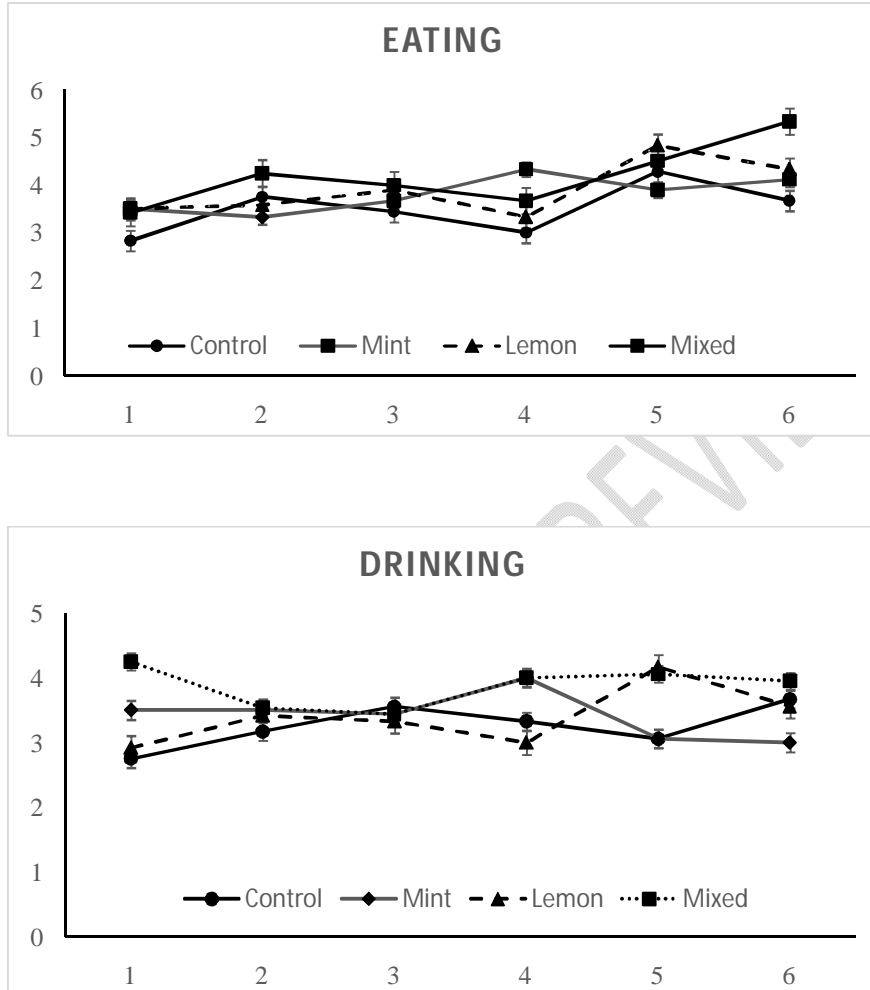


Figure1: Average (Mean±SE) eating and drinking behaviour (N/day) when supplementary Mint, Lemon, and a combination (Mint & Lemon) extraction were added to drinking water (0.3%)