The Effect of Adding Aqueous Mint and Lemon to Heat-Stress Broiler's Drinking Water

ABSTRACT

We aimed to investigate whether adding mint and lemon ingredients would improve broiler performance under heat-stress conditions. A total of 120 - one-day-old, unsexed Hybrid chicks were randomly assigned into four treatment groups, thirty birds each. Each group contained three replicates of ten birds. The experimental groups were assigned according to their watering treatments that, included tape water as the control group, tape water mixed with an aqueous solution (0.3%) of mint (*Mentha longifolia*), tape water with an aqueous solution (0.3%) of lemon (*Citrus limon L*), and tape water with an aqueous mixture (0.3% each) of mint and lemon, respectively. Feed intake and body weight were measured every week. At 42 days of age, two birds per replicate were slaughtered, the carcass was weighed, and its internal organs (chest, thigh, back, wings, neck, liver and abdominal fat) and dressing percentage were calculated. Live observations of drinking and feeding activities were recorded three times a day.

Our results showed that the combination of mint and lemon significantly (P = .05) enhanced the growth performance of chickens (body weight and feed intake), compared to the control, lemons or mint groups, respectively. Carcass and internal organ (Chest, thigh) weights were heavier when lemon was introduced or mixed with a mint than the control and mint groups. Using mint and lemon solely or combined lower abdominal fat weight compared to the control group (P = .05). No significant differences in back, wing, neck, and liver weight were found in treated and untreated groups. Behavioural observation demonstrated that the mint and lemon combination group showed higher drinking and feeding activities than the control, mint and lemon groups. The study concluded that mint and lemon could effectively reduce the impact of high environmental temperatures on chickens. Incorporating mint and lemon into poultry water can improve body weight and reduce carcass fatness.

Keywords: 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 [1]. 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 [2]. Animals suffering from heat stress have been the topic of intensive studies over the last 50 years [3]. 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 [2].

Consequently, there is a growing need to develop and validate reliable behavioural and physiological indicators for heat stress [4]. 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 [5]. Immune function is compromised by various stressors, including poor nutrition, extreme temperatures, injury, and disease [6]. The major problem in the Middle East 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 farming practices, consumers demand healthy food by incorporating alternative methods for using safe substances. Since antiquity, mint (*Mentha longifolia*) has been considered a medicinal herb and a food source. Mint extracts contain hydrocarbons, thymol, and highly oxygenated compounds, which are believed to be responsible for their beneficial characteristics [7]. The composition of essential oils from mint exhibits strong antibacterial [7] and antioxidant activities [7]. Several therapeutic properties are associated with the stems and leaves of mint [8]. Spilling and Daniels [9] report that the leaves contain essential oils that are beneficial to digestion. Mint is positively associated with broilers' growth, as Ocak et al. [10] reported. Mints also support a healthy microbiome in poultry guts [11]. Consequently, the qualities of chilled and frozen broiler meat are improved by supplementation with mint during the rearing process.

The lemon (*Citrus limon L*), a highly phenolic fruit, contains vitamins, minerals, fibre, essential oils, and carotenoids. In animals and humans, these compounds have been shown to increase oxidative resistance, lipid metabolism and digestibility [12]. It was found that dietary supplementation with 1.5% lemon pulp positively affected the reduction of abdominal fat and blood low-density lipoproteins of Ross 308 chicks [13]. The ascorbic acid content in lemon juice obtained by fruit squeezing could enhance chicken growth and metabolism by neutralizing oxygen radicals and reducing oxidative damage [14,15]. Vit C is also a potent scavenger of free radicals in biological systems [14,15]. It assists the body in defending against free radical damage to proteins and lipid membranes and has antioxidant properties. Under heat-stress conditions, broiler chickens were found to have improved immunity when lemon juice was added to their drinking water [16]. It has been demonstrated that Vit C contributes to poultry birds' energy supply by facilitating corticosterone biosynthesis in a heat-stress environment [17]. Vit C has been shown to modify physiological functions and treat and prevent Salmonella [18].

Hence, combining mint and lemon could improve immunity and performance in broilers because their active components have synergistic and suppressive effects. Therefore, our goal in this study was to investigate whether mint (*Mentha longifolia*) and lemon (*Citrus limon L*) can improve broiler chicken production and some performance characteristics (Live and carcass weights, feed, and water intake) in the broiler production cycle and their drinking and feeding behaviour.

2. MATERIAL AND METHODS

2.1 Birds, Diets and Management

A total of 120 - one-day-old, unsexed Hybrid chicks were randomly assigned into four treatment groups, thirty birds each. Each group contained three replicates of ten birds. The experimental groups were assigned according to their watering treatments that, included tape water as the control group, tape water mixed with an aqueous solution (0.3%) of mint (Mentha longifolia), tape water with an aqueous solution (0.3%) of lemon, and tape water with an aqueous mixture (0.3% each) of mint and lemon, respectively.

A commercial vegetable-based feed ration (according to NRC requirements) was provided *ad libitum* during the experiment (Table 1). The chicks were fed a starter (CP: 22%; ME: 3014 kcal) during the first two weeks (days 1-14), a grower (CP: 20 %; ME: 3058 kcal) during (days 15-30), and a finisher diet (CP: 18%; ME: 3115 kcal) during (days 31-42).

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 used during the experimental period. Birds had free access to feed and fresh water throughout the experiment. Feed intake and body weight were measured every week. The amount of food consumed per cage was measured during the experimental period for seven days. Chicks were inspected

daily, and mortality was recorded with the cause of mortality if known. At 42 days of age (end of the experiment), two birds per replicate were slaughtered, the carcass was weighed, and its internal organs (chest, thigh, back, wings, neck, liver and abdominal fat) and dressing percentage were calculated.

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

Diet	Starter 1-14 day	Grower 15-30 day	Finisher 31-42 day
Ingredients and composition			
Yellow corn	61.9	68.5	73.3
Soybean meal (44% CP)	35.5	28.8	24
Dicalcium-phosphate DCP	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
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 amino acid (%)	0.40	0.39	0.34

**: 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. Nutrients available in 100 grams of mint, lemon [19**]**

Component	Mint	Lemon
Water	78.6 g	92.3 g
Energy	293 KJ	91 KJ
Protein	3.75 g	0.35 g
Total lipid (fat)	0.94 g	0.24 g
Ash	1.76 g	0.21 g
Carbohydrate	14.9 g	6.9 g
Calcium, Ca	243 mg	6 mg
Iron, Fe	5.08 mg	0.08 mg
Magnesium, Mg	80 mg	6 mg
Phosphorus, P	73 mg	8 mg
Potassium, K	569 mg	103 mg
Sodium, Na	31 mg	1 mg
Vitamin C	31.8 mg	38.7 mg
Vitamin A, IU	4250 IU	6 IU
Menthone*		20.7–28.8%
Menthol*		19.4–32.5%
Pulegone*		7.8–17.8%
1,8-cineole*		5.6–10.8%
Terpineol-4*		(3.1–4.9%
Piperitone*		2.2–3.3%

** [20]*

Behavioural data were collected using live observations three times a day at 0800, 1200,

and 1500 h, allowing for tabulating the number of chickens eating and drinking in each cage every five minutes during observation hours.

2.2 Preparation of Mint and Lemon Extraction

Mint and lemon aqueous solution was prepared by the soaked three kilograms of fresh mint in warm water (Three litres), covered, and left for 60 minutes, drained, added to six litres of water, and served to birds. Three kilograms of lemon were squeezed, the liquid was collected, and the remaining peel and pulp were air dried, then ground and added to warm water (Three litres) and covered well for 60 minutes. Then, the solution was drained, added to six litres of water, and served to the chickens. The chemical composition of lemon and mint were showed in Table 2.

2.3 Statistical Analysis

This study used a randomized design and an ANOVA involving repeated measures (MIXED model) procedures for analyzing collected data [21]. 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. The model was: $Y_{ijkl} = \mu + T_i + P_j + TP_{ij} + A_k + e_{ijkl}$

Where Y_{ijkl} is the observation value; μ the overall mean; T_i the watering treatment (Tape water vs Tape water with lemon vs tape water with mint vs tape water with lemon-mint mixture); Pj the time period (weeks); TP_{ij} the interaction between treatment and period; A_k the random effect of birds; and e_{ijkl} the residual error. Tukey-Kramer test separated the mean differences among different treatments. A significant level was considered when P <.05. All values were expressed as LSmeans ± SE unless otherwise mentioned.

3. RESULTS

Heat stress interferes with birds' ability to synthesize ascorbic acid. Therefore, the effects of mint and lemon addition to drinking water, either sole or mixed, under heat-stress conditions are shown in Table 3. In the first two weeks, neither the mint nor lemon significantly affected the birds' performance (P > .05). In contrast, the mixed combination of mint and lemon improved feed intake, body weight, and feed conversion ratio (P < .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 < .05).

Table 3. The effect of water supplement with aqueous extract (0.3%) of mint and lemon on body weight and feed intake of heat-stressed chicks. *

Age (day)	Weight (g)	Control (Tape water)	Mint (Tape water with 0.3 % mint)	Lemon (Tape water with 0.3 % lemon)	Mint & Lemon (Tape water with mint 0.3% and lemon 0.3 %)
1-7	BW	157.93 ± 5.07	161.88 ± 5.07	160.05 ± 5.07	162.48 ± 5.07
	FI	137.60 ± 2.49	137.33 ± 2.49	137.33 ± 2.49	147.33 ± 2.49
7-15	BW	364.97 ± 5.07	365.87 ± 5.07	365.22 ± 5.07	367.80 ± 5.07
	FI	249.57 ± 2.49^{B}	264.33 ± 2.49^{Ad}	260.33 ± 2.49^{Bd}	282.00 ± 2.49^{Ac}
15-21	BW	672.67 ± 5.07^{B}	681.83 ± 5.07^{Bd}	683.27 ± 5.07^{Bd}	711.63 ± 5.07^{Ac}
	FI	524.93 ± 2.49^{B}	542.00 ± 2.49^{Ac}	526.00 ± 2.49^{Bd}	547.67 ± 2.49^{Ac}
21-28	BW	1158.97 ± 5.07^{B}	1160.67 ± 5.07^{Bc}	1183.27 ± 5.07^{Bc}	1206.20 ± 5.07^{Ac}
	FI	808.33 ± 2.49^{B}	806.00 ± 2.49^{Ad}	802.00 ± 2.49^{Ae}	835.00 ± 2.49^{Ac}
28-35	BW	1505.10 ± 5.07^{B}	1527.37 ± 5.07^{Be}	1569.43 ± 5.07^{Ad}	1622.87 ± 5.07^{Ac}
	FI	823.67 ± 2.49^{B}	868.00 ± 2.49^{Ad}	853.33 ± 2.49^{Ae}	924.67 ± 2.49^{Ac}
35-42	BW	2057.10 ± 5.07^{B}	2117.97 ± 5.07^{Ae}	2216.80 ± 5.07^{Ad}	2277.77 ± 5.07^{Ac}
	FI	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 =.05*

** $^{A, B}$: Treatment differences (control vs treatment addition)*

** ^{c,d,e}: Differences within the treatments (mint vs lemon vs mint and lemon combination)*

Carcass and eviscerated yields increased in weight as the body weight increased. As shown in Table 4, carcass and thigh muscles were heavier than the control and mint group (p < .05) when birds drank water either with lemon or combined with mint and lemon. Furthermore, water treatments showed significantly higher chest weight and lower abdominal fat weight

compared to the control group (P < .05). Back, wing, neck, and liver weights didn't show any significant differences in treated and untreated groups (P > .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 (Fig. 1). Chicks in mint and lemon mixture treatment showed higher drinking (3.87 N /day) and feeding (4.20 N / day) activities compared to the chicks in mint (3.42 and 3.81N / day) and lemon (3.40 and 3.91 N / day) groups for drinking and feeding respectively.

4. DISCUSSION

Heat stress has been demonstrated to negatively impact bird welfare and meat quality [22-24]. The heavy feathers on poultry prevent them from sweating and releasing heat into the environment through their body feathers. Therefore, heat-stressed broilers perform several physiological and behavioural mechanisms to cope with the hot climate by reducing feed consumption and activity, increasing water intake and body temperature, resting during the heat stress, and spreading their wings and pant to promote cooling [25]. Reduced feed intake is presumed to contribute to the most detrimental effects of heat-stressed birds' productivity [3]. As a result of panting, the acid-base balance of birds is disturbed, which increases the amount of oxidative damage. Abidin and Khatoon [17] reported that birds' plasmas had lower antioxidant vitamin C concentrations, potentially increasing oxidative damage. Due to this, reducing the adverse effects of elevated temperatures requires inhibiting stress production. In parallel with our findings, Spirling and Daniels [9] found that mint positively affects digestion and feed intake due to its pharmacological properties. It enhances intestinal function, bile production, microbial growth, bacteria formation, erratic activity, and choleretic activity [26,27]. As a result of the addition of lemon to poultry diets, body weight tends to increase, mortality is reduced, performance is improved, feed intake is increased, and feed efficiency is improved [17]. Due to its active ingredient, Vitamin C, lemons enhance the immunity of broiler chickens and protect them against reactive oxygen species (ROS) forming during high temperatures [16]. Vitamin C stimulates the production of corticosterone, which helps poultry maintain their energy supply during hot weather [16].

Table 4. Average live carcass weight (g/bird) and internal viscera when supplementary aquoues extarct (0.3%) mint, lemon, and a combination of mint and lemon were added to drinking water*

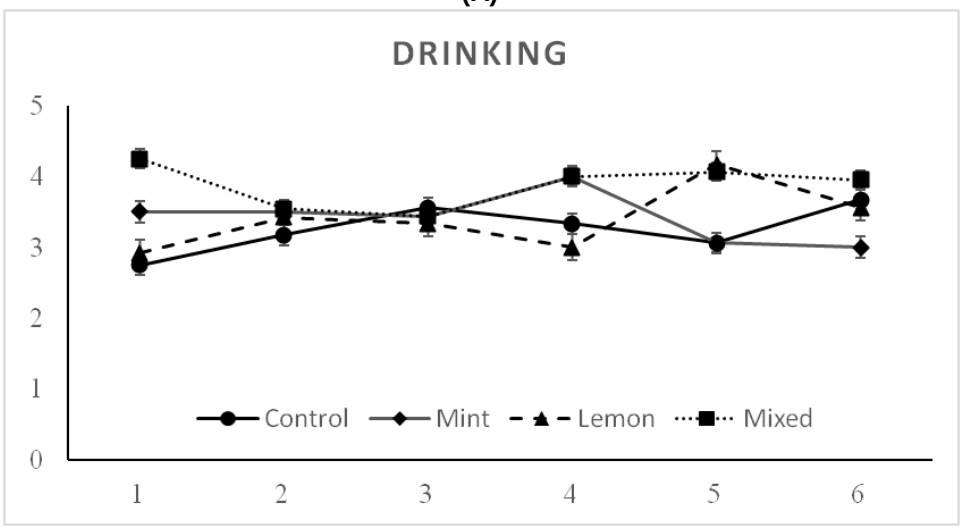
Weight (g)	Control	Mint (0.3%)	Lemon (0.3%)	Mint & Lemon
Live	2078.17 ± 23.83^{B}	2144.17 ± 23.83^{Bd}	2189.67 ± 23.83^{Ac}	2253.87 ± 23.83^{Ac}
Carcass	1521.30 ± 20.37^{B}	1592.84 ± 20.37^{Bd}	1632.94 ± 20.37^{Acd}	1695.11 ± 20.37^{Ac}
Chest	32.48 ± 0.25^{B}	35.72 ± 0.25^{Ac}	35.85 ± 0.25^{Ac}	36.67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

** A row of values with different superscripts differs significantly at P =.05*

** $^{A, B}$: Treatment differences (control vs treatment addition)*

** ^{c,d,e}: Differences within the treatments (mint vs lemon vs mint and lemon combination)*

(A)



(B)

Fig. 1(A-B). Average (Mean±SE) eating and drinking behaviour (N/day) when supplementary aquoues extarct (0.3%) mint, lemon, and a combination of mint and lemon were added to drinking water*

The chemical components of mint are menthol, menthone, 1,8-cineole, methyl acetate, methofuran, isomenthone, limonene, b-pinene, a-pinene, germacrene-d, trans-sabinene hydrate, and pulegone, which are believed to be responsible for their beneficial characteristics [20]. Among the main phenolic components of mint oil, menthol has antibacterial properties. It has been demonstrated that this plant has antiseptic, spasmolytic, and disinfectant properties [11]. 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. Therefore, adding a mixture of 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. Thus, combining mint and lemon has successfully enhanced broilers' feed intake, resulting in improved performance. Fat deposition is considered a negative feature since it represents a nutritional loss; it may also contribute to decreased carcass yields and meat consumption [28]. Thus, mint and lemon might enhance fatty acid metabolism through improved digestion and choleretic effects during digestion by reducing hepatic lipogenesis and stimulating fatty acid metabolism.

Drinking and feeding behaviour showed that once the feed is consumed, the birds spend their time drinking, which reflects the higher feeding

and drinking behaviour of chickens that receive a combination of mint and lemon in their water that boosts the immune system, increases feed intake and promotes micronutrient absorption.

5. CONCLUSION

In conclusion, using mint (menthol) and lemon (Vit C) as their active components synergize effectively alleviates the harmful impacts of high temperatures on broiler chickens. Consequently, adding mint and lemon to poultry water can improve body weight and reduce carcass fatness.

ETHICAL APPROVAL

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

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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