Evaluation of Essential Oil Products on Performance, Intestinal Permeability, Bone Mineralization, and Oxidative Stress Parameters on Broiler Chickens Under Heat Stress Conditions

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Abstract

The goal of this study was to show how dietary supplementation of three different formulations of essential oils (EO) affected the performance of broiler chickens during heat stress (HS). Day-of-hatch Cobb 500 chicks (n = 500) were randomly divided into four groups: 1. HS control + control diets; 2. HS + control meals supplemented with 37 ppm EO of *Lippia origanoides* (LO); 3. HS + control diets supplemented with 45 ppm LO + 45 ppm EO of *Rosmarinus officinalis* (RO) + 300 ppm red beetroot; 4. HS + 45 ppm LO + 45 ppm RO + 300 ppm natural betaine. When compared to control HS chickens, the EO chickens had a substantial ($p \leq 0.05$) improvement in BW, BWG, FI, and FCR. Average body core temperature in group 3 and group 4 was significantly ($p \leq 0.05$) reduced compared with the HS control group and essential oil group 2. When compared to the HS control, experimental groups demonstrated a substantial reduction in FITC-d at 42 days, a significant rise in SOD at both days, but a significant loss in IFN-γ and IgA ($p \leq 0.05$). Treating birds with EO enhanced bone mineralization considerably ($p \leq 0.05$). These findings imply that dietary EO supplementation may help to minimize the adverse effects of HS.

Keywords: broiler chickens; essential oils; heat stress; *Lippia origanoides*; *Rosmarinus officinalis*

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Chapter 1. Introduction

In previous decades, there has been an increase in environmental temperatures and intensification of weather patterns on a global scale (Diffenbaugh & Ashfaq, 2010). Since 1880, Carbon dioxide (CO2) concentrations globally have increased from 290 parts per million (ppm) to 430 ppm (Henderson et al., 2018). Carbon dioxide absorbs radiation from the sun causing a higher global temperature. Animals such as poultry, lack sweat glands and efficient cooling mechanisms, making them especially susceptible to these extreme temperatures (Abu-Dieyeh, 2006). Along with the growing concern of environmental extremes, the poultry industry is aware of antibiotic residues and antibiotic resistance with alternative growth promoters (AGP). Essential oils (EO) naturally occur in plants as secondary metabolites and have been displayed commercially in livestock production to be a more natural AGP. In several studies, EO as feed additives have shown to have antibacterial, antiviral, antifungal, and antioxidant properties. They have also exhibited other properties such as: digestive stimulants, immunomodulators, hypolipidemic agents, to alleviate heat stress in poultry (Turcu et al., 2018; Zhai et al., 2018; Saadat Shad et al., 2016; Patra, 2020; Zeng et al., 2015).

Heat stress (HS) as defined by Abu-Dieyeh, Z. (2006) is a failure to thermoregulate normal body temperature. Heat stress impacts the welfare of birds leading to economic loss. In 2017, it was estimated that HS amounted to a devastating 2.36 billion dollars in economic losses of livestock annually in the U.S. (Polsky & Keyserlingk, 2017). Birds suffering immense thermal stress also experience oxidative stress, triggering separation of the tight junction proteins in the epithelial layer of the intestine. This leads to a decrease in body weight gain and feed intake as the birds switch from thriving to surviving (Andersen et al., 2017). With the physiological transition to a survival mode, certain systems, such as reproductive, renal, and respiratory, are greatly impaired in an effort to avoid producing any more heat.

In an attempt to reduce the carbon footprint of production, researchers are constantly looking to find a more natural alternative to AGP that enhances welfare and reduces the negative effects of HS. Dietary EO have been proposed by the Poultry Health Laboratory as a solution to these problems facing our industry. Given the bactericidal properties of EO, this has been all the more reason to investigate their inclusion as alternatives to AGP (Wallace et al., 2010). Effectively incorporating EO in poultry nutrition to improve the health of the birds and reduce economic losses could be a major victory for the poultry industry in the U.S. and abroad. Many other countries do not have the same access to equipment that growers use in the U.S regarding ventilation, evaporative cooling cells, tunnel fans, and consistent electricity. Not only is HS harmful to the birds, but it has the potential to lessen the quality and quantity of food for people (Quinteiro-Filho et al., 2010). Incorporating dietary supplements such as EO improves the quality of life for the birds and results in improved meat yield (Furlan et al., 2000). The purpose of the present study is to assess dietary supplementation of three EO blends on the performance, intestinal permeability, bone mineralization, antioxidant, and anti-inflammatory parameters in broiler chickens under cyclic heat stress conditions.

Chapter 2. Literature Review

This chapter provides a perspective on the gastrointestinal, physiological, and financial impact that HS has on broiler chickens. Heat stress has been shown to affect many environmental and biological organisms. Animals without sweat glands, such as chickens are especially susceptible to HS and its adverse effects (Altan et al., 2003). On the molecular level, this discussion will cover how the thermal stress triggers oxidative stress and how this physiologically impacts the epithelial layer of the intestine. In several studies, EO have been shown to have more natural growth promoting properties (Proestos et al., 2013; Alavi et al., 2011). This chapter will conclude with review of studies demonstrating the effectiveness of beetroot and betaine in livestock diets as well as how they improve health and growth.

Economic and Physiological Impacts of Heat Stress in Broilers

One of the most common stressors on livestock around the world occurs through the weather and environment. The majority of poultry production in the Unites States takes place in the southern part of the U.S where the climate tends to be warmer, which makes mitigative strategies for HS all the more necessary. When HS is applied to broilers there is a decrease in feed intake, body weight, and body weight gain (Quinteiro-Filho et al., 2010). The economic loss incurred is understandable after considering how HS affects body weight and feed intake, as approximately 80% of cost of poultry production is in the feed. If feed is being wasted as a result of not being consumed or not being efficiently converted to muscle, growers and integrators may suffer economically. A study by Owens et al. (2000) found that in broiler chickens there was a high correlation between heat stress and pale, exudative meat. Economically, this is concerning given that birds sent for processing after significant HS exposure may exhibit myopathies or be discarded by USDA standards due to undesirable characteristics of pale, exudative meat.

When birds experience thermal stress, they are only concerned with avoiding producing more heat (Hoover, 2020). Because avian species lack sweat glands and have feathers for warmth, it can be challenging to thermoregulate. Panting (a mechanism for cooling) for an extended amount of time can lead to an acid-base imbalance and respiratory alkalosis which can seriously impair the birds, if not kill them (Lara et al., 2013). Chicks at hatch are unable to regulate their own body temperature yet, have been observed to acclimate easily to higher temperatures (Rostagno, 2013). However, older birds around 28 days and beyond are most susceptible to HS and the related harmful effects (Cooper & Washburn, 1998). Furthermore, HS decreases body weight and increases mortality (Haldar et al., 2011). This has behavioral, physical, economical, and social consequences for the poultry industry.

Heat Stress and Gut Health

The gastrointestinal tract is often referred to as the second brain. When this organ is impaired, immune, hormonal, digestive, reproductive, and physical functions of the body can be inhibited. Heat stress is a mechanism that can impair the gastrointestinal tract (Kang & Shim, 2020). According to Mahmoud et al. (2020), one of the functions of the epithelium layer is to protect from harmful bacteria and toxins that could translocate across the barrier. This barrier is held together by tight junction proteins. When these tight junction proteins become disrupted harmful microorganisms are able to easily translocate across the barrier causing systemic inflammation and possibly infection (Mahmoud et al., 2020). When reactive oxygen molecules are accumulated and cells are unable to detoxify the production of free radicals and reactive oxygen species this is known as oxidative stress (Altan et al., 2003). Oxidative stress can be triggered by a variety of factors including biological, chemical, emotional, and physical (Sahin et al., 2017). This oxidative stress triggers harmful free radicals. Those free radicals could be reactive oxygen, nitrogen, or chlorine molecules. When free radicals trigger cytokine production, they induce inflammation of the intestine and disrupt the tight junction proteins making the gastrointestinal tract susceptible to disease and poor performance (Tekce et al., 2016).

Essential Oils

In general, there is rising pressure from consumers to move away from the use of antibiotics and AGP in the poultry industry in recent decades. Several companies and integrators in the livestock industry have examined EO for a variety of positive effects when supplemented at the right inclusion. EO are found naturally as secondary metabolites in plants (Turcu et al., 2018). Several studies showed that EO's can improve digestive stimulation, antioxidant, anti-inflammatory, enzyme secretion, and immune response properties in chickens (Habibi et al., 2014; Sahin et al., 2017; Wen et al., 2019). Dietary betaine has been used and supplemented in a variety of EO research diets (He et al., 2015; Wen et al., 2019; He et al., 2015), and been shown to lower FCR and increase feed intake while increasing fat metabolism. An increase in fat metabolism decreases the amount of fat deposits in the subcutaneous, abdominal, intramuscular, and lipid cholesterol concentrations allowing the broilers to have better meat quality and dissipate heat easier (Lidder & Webb, 2013).

In summary, heat stress can have devastating pathogenic consequences on broiler health. In an effort to lower carbon emissions and find an effective replacement for current alternative growth promoters, essential oils have become a forerunner as a new dietary supplement to lessen the negative effects that heat stress has on poultry.

Chapter 3: Materials and Methods

This chapter discusses the protocol, procedures, and sampling methods performed in this study. The experiment is divided into two sections. The first section describes procedures used in raising Cobb 500 Broilers. The second part is on the laboratory tests and analyses of data collected from production. All production methods follow Cobb 500 industry standards that are outlined in the paragraphs below. The purpose of this research was to determine the effectiveness of dietary EO blends on broiler chickens raised in a heat stress environment in an attempt to mitigate the negative outcomes associated with heat stress.

Research Design

The experiment was a quantitative, true experimental design with random assignment, and replication. This design was justified based on the Poultry Science Association guidelines for research (Poultry Science, 2017).

Methodology

This study was conducted at the Poultry Experimental Research Laboratory (PERL) facility at the University of Arkansas. The PERL has individual environmentally controlled rooms equipped with its own air handling unit and digital thermostat to control temperature. Day-of-hatch Cobb 500 male broiler chicks (n= 500) were obtained from a commercial hatchery. Upon arrival, all chickens were vaccinated with a coccidia vaccine (Coccivac®-B52, Merck Animal Health, De Soto, KS 66018), neck tagged, and randomly distributed into four groups. Group 1: HS control + control diets; Group 2: HS + control diets supplemented with 37 ppm EO of *Lippia origanoides* (LO); Group 3: HS + control diets supplemented with 45 ppm LO + 45 ppm EO of *Rosmarinus officinalis* (RO) + 300 ppm beetroot; Group 4: HS + 45 ppm LO + 45 ppm RO + 300 ppm Natural Betaine. The starter, grower, and finisher diets used in this research were adjusted to broilers' recommendations (Cobb-Vantress, 2018) and formulated to provide an adequate supply of nutrients (Table 1). No growth promoters were included in the diets. Diets were formulated to be isocaloric and isonitrogenous across all phases. Groups were allocated to ten environmental rooms. Each room was divided into two pens (150 X 300 cm), each containing separate feeders and watering systems, five replicates per treatment with 25 birds/replicate (n=125). At placement, chickens were received with 34°C and relative humidity at 55 ± 5% for the first 7 d. During cyclic HS, chickens received 35°C for 12 h daily from day 7 to day 42. Relative humidity remained constant at 55 ± 5%. On d 18, eight chickens were randomly selected to orally insert a Thermochron temperature logger (iButton, DS1922L, Embedded Data Systems, Lawrenceburg, KY). The devices stayed in the gizzard for measurement of body temperature, as described by Flees et al., 2017. Every minute during the first two hours after initiation of heat stress and every subsequent hour, the chickens' body temperature was logged (n=24 chickens). Performance parameters such as body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were evaluated at d 21 and 42. On the same days, four random chickens per pen were selected (n= 20) and orally gavaged with 8.32 mg/kg of body weight of fluorescein isothiocyanate-dextran (FITC-d, MW 3-5 KDa; Sigma-Aldrich Co). One hour after FITC-d administration, chickens were humanely euthanized by CO_2 inhalation. Blood samples were collected from the femoral vein and centrifuged (1000×g for 15 min) to separate the serum. Serum levels of FITC-d were used as a biomarker to evaluate intestinal permeability as described by Baxter et al (2017). From the same serum samples, commercial kits were used to evaluate superoxide dismutase (SOD), gamma interferon (IFN-γ), and immunoglobulin A (IgA) as described by Baxter et al. (2019). The left and right tibias from each sampled chicken on days 21 and 42 were removed to assess break strength (kg) and total ash based on fat-free tibia (%) as described by Gautier et al. (2017). On day 42, nine chickens per replicate pen (n= 45 HS) per group were selected to evaluate processing parameters. Animal handling procedures followed the Institutional Animal Care and Use Committee at the University of Arkansas under protocol number 16084.

Determination of bone parameters

The bones were held in identical positions and the mid-diaphyseal diameter of the tibial midshaft, which was also the site of impact, was measured using a dial caliper. The maximum load at failure was determined in the tibial midsection between epiphyses, using a three-point flexural bend fixture with a total distance of 30 mm between the two lower supporting ends. The load, defined as the force in kilograms per square millimeter of cross-sectional area (kg/mm2), represents bone strength. The rate of loading was kept constant at 20 mm/min collecting 10 data points per second. The data were automatically calculated using Instron's Series IX Software (Norwood, MA). Tests were performed at the University of Illinois.

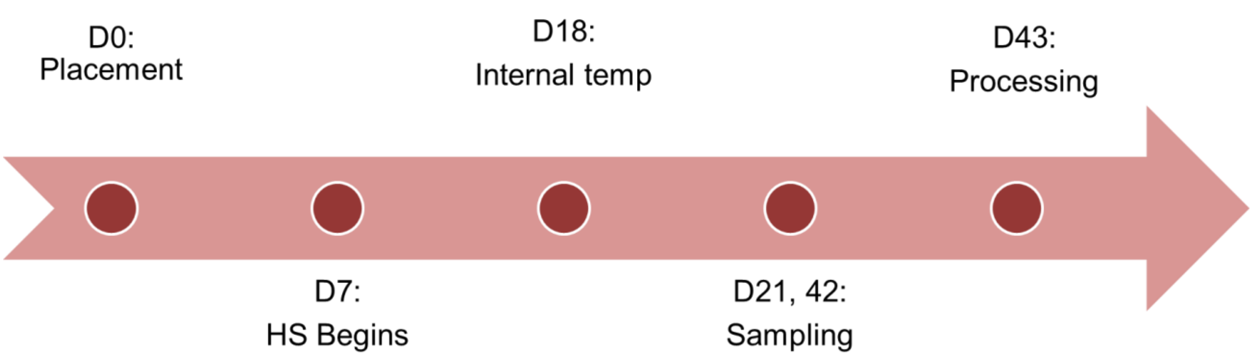

Figure 1. Experimental Schedule

Table 1. Ingredient composition and nutrient content of the corn-soybean diet used on an as-is basis.

Feed ingredients	Stater phase (d 1 to 7)	Grower phase (d 8 to 14)	Finisher phase (d 15 to 25)
Ingredients (%)			
Corn	51.80	57.81	59.64
Soybean meal	37.66	31.62	27.23
DDGS 8.1% EE	4.00	4.00	6.00
Poultry fat	3.24	3.44	4.38
Limestone	1.01	1.06	1.03
Dicalcium phosphate	1.00	0.88	0.64
Salt	0.35	0.35	0.31
DL-methionine	0.29	0.31	0.28
L-lysine HCl	0.12	0.13	0.12
Mineral premix[b]	0.10	0.10	0.10
Vitamin premix[a]	0.10	0.10	0.10
L-threonine	0.08	0.09	0.09
Choline chloride	0.06	0.06	0.05
Sodium bicarbonate	0.04	0.05	0.03
Antioxidant[c]	0.15	0. 15	0. 15
Total	100	100	100
Calculated analysis			
ME (kcal/ kg)	3015.00	3090.00	3175.00
Ether extract (%)	5.88	6.20	7.28
Crude protein (%)	22.30	20.00	18.70
Lysine (%)	1.18	1.05	0.95
Methionine (%)	0.59	0.53	0.48
Threonine (%)	0.77	0.69	0.65
Tryptophan (%)	0.25	0.22	0.20
Total calcium (%)	0.90	0.84	0.76
Total phosphorus (%)	0.63	0.58	0.53
Available phosphorus (%)	0.45	0.42	0.38
Sodium (%)	0.20	0.20	0.18
Potassium (%)	1.06	0.94	0.87
Chloride (%)	0.27	0.28	0.25
Magnesium (%)	0.19	0.18	0.17
Copper (%)	19.20	18.46	18.85
Selenium (%)	0.28	0.27	0.26
Linoleic acid (%)	1.01	1.13	1.16

[a] Vitamin premix supplied the following per kg: vitamin A, 20,000,000 IU; vitamin D3, 6,000,000 IU; vitamin E, 75,000 IU; vitamin K3, 9 g; thiamine, 3 g; riboflavin, 8 g; pantothenic acid, 18 g; niacin, 60 g; pyridoxine, 5 g; folic acid, 2 g; biotin, 0.2 g; cyanocobalamin, 16 mg; and ascorbic acid, 200 g (Nutra Blend LLC, Neosho, MO 64850).
[b] Mineral premix supplied the following per kg: manganese, 120 g; zinc, 100 g; iron, 120 g; copper, 10–15 g; iodine, 0.7 g; selenium, 0.4 g; and cobalt, 0.2 g (Nutra Blend LLC, Neosho, MO 64850).
[c] Ethoxyquin.

Data analyses

All data were subjected to analysis of variance as a completely randomized design, using the General Linear Models procedure of SAS (SAS Institute, 2002). Significant differences among the means were determined by Duncan's multiple range test at $P < 0.05$.

Chapter 4. Results

The results of the evaluation of EO on broiler chickens exposed to cyclic HS on performance parameters and carcass component weights are summarized in Table 2. Chickens that received the EO showed significant ($P \leq 0.05$) improvement on BW, BWG, FI, and FCR compared to control HS chickens (Table 2). Cyclic HS reduced all parameters evaluated for carcass component weights (hot carcass, chilled carcass, wing, breast, tender, and leg and quarter). Interestingly, in the present study, the formulations of EO in group 3 (supplemented with 45 ppm *Lippia origanoides* + 45 ppm Rosmarinus officinalis + 300 ppm beetroot) and group 4 (supplemented 45 ppm *Lippia origanoides* + 45 ppm *Rosmarinus officinalis* + 300 ppm natural betaine) significantly mitigated the harmful effects of HS in carcass component weights when compared with the HS control chickens (Table 2). Carcass components weights of birds in group 2 (LO) were however not significantly different from the HS control group.

Table 2. Evaluation of essential oils on broiler chickens exposed to cyclic heat stress on performance parameters and carcass component weights at days 21 and 42.

	Heat stress control	***Lippia origanoides***	***L. origanoides*, R. officinalis,* Beetroot**	***L. origanoides*, R. officinalis,* Natural betaine**	**Pooled SEM**	***P*- value**
BW, g/broiler						
d 0	43.62	43.20	43.71	43.82	0.98	0.1457
d 21	612.30 ^{b}	689.41 ^{a}	680.24 ^{a}	695.20 ^{a}	28.90	0.0002
d 42	2,119.20 ^{c}	2,329.90 ^{ab}	2,242.11 ^{ab}	2,380.75 ^{a}	125.42	0.0001
Accumulated BWG, g/broiler						
d 0 to 21	569.30 ^{b}	646.41 ^{a}	637.24 ^{a}	652.20 ^{a}	26.78	0.0001
d 0 to 42	2,016.20 ^{c}	2,286.90 ^{ab}	2,199.11 ^{ab}	2,337.75 ^{a}	119.87	0.0002
FI, g/broiler						
d 0 to 21	790.30 ^{b}	910.41 ^{a}	891.24 ^{a}	930.20 ^{a}	32.40	0.0001
d 0 to 42	4,110.20 ^{c}	4,355.90 ^{ab}	4,125.11 ^{bc}	4,284.75 ^{a}	230.56	0.0002
Accumulated FCR						
d 0 to 21	1.30	1.32	1.31	1.33	0.87	0.1689
d 0 to 42	1.94 ^{a}	1.87 ^{b}	1.84 ^{b}	1.80 ^{b}	0.92	0.0001
Carcass component weights (g) at day 42						
Live weight	2156.25 ^{c}	2240.91 ^{bc}	2361.15 ^{ab}	2423.30 ^{a}	201.36	0.0001
Hot carcass	1640.88 ^{c}	1688.86 ^{bc}	1788.49 ^{ab}	1836.98 ^{a}	187.32	0.0002
Chilled carcass	1687.38 ^{b}	1731.16 ^{b}	1847.51 ^{a}	1885.35 ^{a}	198.86	0.0001
Wing	180.85 ^{c}	183.57 ^{bc}	189.55 ^{ab}	196.83 ^{a}	20.13	0.0001
Breast	330.02 ^{b}	336.30 ^{b}	371.98 ^{a}	380.65 ^{a}	31.10	0.0001
Leg and quarter	534.40 ^{c}	548.98 ^{bc}	579.13 ^{ab}	588.65 ^{a}	42.02	0.0002

**L. origanoides* (45 ppm); *R. officinalis* (45 ppm); Beetroot (300 ppm); Natural betaine (300 ppm). Data are expressed as the mean ± SE. Different superscripts indicate [a-c] significant differences between the treatments within the rows ($P \leq 0.05$).

The evaluation of essential oils on broiler chickens exposed to cyclic heat stress on body core temperature, serum biomarkers for intestinal inflammation, and bone parameters at days 21 and 42 is summarized in Table 3. Only two hours after introducing HS in the experimental groups, a significant increase ($P \leq 0.05$) in the body core temperature of the chickens was observed and heightened body temperature during heat stress was observed through the trial (data not shown). Average body core temperature in group 3 (supplemented with 45 ppm *Lippia origanoides* + 45 ppm *Rosmarinus officinalis* + 300 ppm beetroot) and group 4 (supplemented 45 ppm *Lippia origanoides* + 45 *ppm Rosmarinus officinalis* + 300 ppm natural betaine) was significantly reduced when compared with HS control group and group 2, supplemented with 37 ppm EO of *Lippia origanoides* (Table 3). At 21 days, only groups 3 and 4 showed a significant reduction in serum FITC-d, the intestinal permeability biomarker. However, all experimental groups that were treated showed a significant reduction in serum FITC-d at 42 days compared with control HS chickens. In the present study, experimental treated chickens had a significant increase ($P \leq 0.05$) in serum concentrations of SOD at both days of evaluation compared to control HS chickens, but had significant reduction ($P \leq 0.05$) in serum levels of gamma interferon and IgA (Table 3). All three experimental groups showed a significant increase ($P \leq 0.05$) in tibia break strength at both days of evaluation, however, total ash from tibia was significantly higher ($P \leq 0.05$) in groups 3 and 4 at 21 and 42 days of evaluation (Table 3).

Table 3. Evaluation of essential oils on broiler chickens exposed to cyclic heat stress on body core temperature, serum biomarkers for intestinal inflammation, and bone parameters at days 21 and 42.

	Heat stress control	***Lippia origanoides***	****L. origanoides, R. officinalis,*** **Beetroot**	****L. origanoides, R. officinalis,*** **Natural betaine**	**Pooled SEM**	***P*- value**
Body core temperature (°C)	42.36 ^{a}	42.35 ^{a}	41.98 ^{b}	41.98 ^{b}	0.83	0.0001
Serum FITC-d (ng/mL)						
d 21	264 ^{a}	288 ^{a}	152 ^{b}	251 ^{b}	95	0.0001
d 42	245 ^{a}	165 ^{b}	137 ^{bc}	129 ^{c}	82	0.0002
SOD (U/mL)						
d 21	7.35 ^{b}	8.66 ^{a}	8.55 ^{a}	9.01 ^{a}	0.45	0.0001
d 42	8.45 ^{b}	9.73 ^{a}	10.05 ^{a}	10.85 ^{a}	0.61	0.0002
IFN-γ (pg/ml)						
d 21	134 ^{a}	118 ^{b}	112 ^{b}	116 ^{b}	17	0.0001
d 42	251 ^{a}	131 ^{b}	122 ^{b}	133 ^{b}	22	0.0002
IgA (ng/mL)						
d 21	14 ^{a}	8 ^{b}	9 ^{b}	8 ^{b}	0.38	0.0001
d 42	16 ^{a}	9 ^{b}	10 ^{b}	9 ^{b}	0.53	0.0001
Tibia break strength (kg)						
d 21	13.79 ^{b}	15.69 ^{a}	16.09 ^{a}	15.99 ^{a}	1.12	0.0001
d 42	22.37 ^{b}	29.17 ^{a}	30.37 ^{a}	31.37 ^{a}	2.05	0.0002
Total ash from tibia (%)						
d 21	50.57 ^{b}	51.67 ^{b}	52.67 ^{a}	53.77 ^{a}	0.49	0.0001
d 42	52.33 ^{b}	53.34 ^{ab}	54.34 ^{a}	55.04 ^{a}	0.31	0.0001

**L. origanoides* (45 ppm); *R. officinalis* (45 ppm); Beetroot (300 ppm); Natural betaine (300 ppm). Data are expressed as the mean ± SE. ^{abc} Indicates significant differences between the treatments within the rows (P ≤ 0.05).

Chapter 5: Discussion and Conclusions

Discussion

Maintaining good bird health is essential for better resistance to the physiological difficulties associated with HS. To minimize the negative consequences of HS in poultry, several management and nutritional methods have been explored, with plant-based additions showing potential (Saadat Shad et al., 2016). Because of their antibacterial, antiviral, antifungal, antioxidant, immunomodulatory, hypolipidemic, and heat stress alleviating properties, EO have attracted special attention as natural options for substituting AGPs in chicken diets (Habibi et al., 2014; Sahin et al., 2017; Wen et al., 2019). These EO have been demonstrated to improve nutrient absorption as well as productive and reproductive performance when used as feed additives (Patra, 2020). In this project, groups that received EO and were subjected to cyclic HS had higher BW and BWG at 21 and 42 days compared to HS control chickens (d 42 only). These findings are consistent with earlier studies (Habibi et al., 2014; Sahin et al., 2017; Wen et al., 2019) that have demonstrated HS chickens receiving EO from oregano, rosemary, or betaine all had improved performance. Other research suggests that the improved performance shown in poultry fed EO is related to the stability of microbial eubiosis in the gut, enhanced digestive enzyme production, and stimulated hunger (Patra, 2020).

Heat stress exerts substantial metabolic and physiological consequences in contemporary broiler chickens, including decreased gene expression of lipoprotein lipase and hepatic triacylglycerol lipase and increased gene expression of adipose triglyceride lipase (Tekce and Gül, 2016). These alterations in gene expression are linked to an increase in abdominal, intermuscular, and subcutaneous tissue fat deposition in HS-affected chickens. Furthermore, HS causes cellular osmosis and dehydration, which results in increased plasma lipid and glucose, serum calcium (due to bone demineralization), and total blood protein. These changes have a major negative impact on the water holding capacity of chicken meat products (Rajaei-Sharifabadi et al., 2017), which has direct impact on meat texture.

The findings of this project support an earlier study that showed HS weakened the intestinal barrier and increased gut permeability (Ruff et al., 2020). On day 21 of the study, EO formulations in groups 3 and 4 showed a substantial decrease in serum FITC-d compared to the other groups exposed to HS. However, after 42 days of testing, all three EO formulations in chickens subjected to cyclic HS significantly decreased FITC-d leakage when compared to control HS chicks. FITC-d is a big molecule (3-5 kDa) that generally does not pass through the intact gastrointestinal tract barrier. However, when conditions disrupt the tight junctions between epithelial cells, the FITC-d molecule can enter circulation, as demonstrated by an increase in transmucosal permeability associated with chemically induced disruption of tight junctions by elevated serum levels of FITC-d after oral administration (Baxter et al., 2017). According to a 2020 study by Patra, EO reduces intestinal permeability by raising the gene expression of tight junction (TJ) proteins, decreasing the gene expression of proinflammatory cytokines, and boosting the proliferation of goblet cells.

Arguably, the most researched EO characteristics include antioxidant activity, radical scavenging, and antibacterial activities (Mehmood et al., 2013). The antioxidant systems of chickens are in a state of dynamic equilibrium under normal temperature conditions and can respond to typical challenges associated with broiler production. During HS, however, reactive oxygen species are generated at quantities that are greater than the system's ability to tolerate, resulting in oxidative stress (Altan et al., 2003). Superoxide dismutases (SOD) are enzymes that convert superoxide free radicals into hydrogen peroxide and molecular oxygen (Zeng et al., 2015). In this study, all three dietary formulations of EO examined elevated serum SOD concentrations on days 21 and 42 when compared to the HS control chickens. The ability to scavenge free radicals safeguards the integrity of cellular and mitochondrial membranes against lipid peroxidation (Proestos et al., 2013; Alavi et al., 2011). Essential oils of rosemary, oregano, thyme, and turmeric enhanced the antioxidant response in oxidatively stressed enterocytes, indicating a novel mechanism by these agents. Other researchers have reported the antioxidant activity of Lippia (Stashenko et al., 2008), rosemary (Gachkar et al., 2007), and beetroot (Gachkar et al., 2007; Guldiken et al., 2016).

The negative effects of prolonged HS also include an increase in respiration rate causing respiratory alkalosis. In addition to raising carbonyl concentrations and thiobarbituric acid reactive substances (TBARS) because of lipid peroxidation (Rostagno, 2013), respiratory alkalosis also affects chicken meat quality by lowering the water content and changing the color of the breast muscle. Other studies have found that chronic HS has a negative impact on carcass yield and meat quality (Alirezaei et al., 2012; McDevitt et al., 2000; Aditya et al., 2018). Interestingly, when compared to HS control birds, chickens in groups 3 and 4 exhibited substantial gains in carcass component weights in the current research. Both groups used a combination of EO and a supply of the essential proteinogenic amino acid betaine, commonly known as the "carcass modifier" due to its osmoprotective and osmoregulatory characteristics in cells, particularly under HS circumstances (He et al., 2015).

The antioxidant, anti-inflammatory, and apoptotic characteristics of betalain pigments in beetroot (Beta vulgaris) make it particularly appealing (Guldiken et al., 2016). Recently, the high content of nitrate (NO3-) in beetroot has been related to endogenous nitric oxide generation (NO). This has been linked to participation in vascular, inflammatory, apoptotic, and neurotransmission responses, which have gotten a lot of attention throughout the world (Lidder & Webb, 2013). Surprisingly, poultry subjected to cyclic HS and given the EO formulations had much lower blood amounts of the proinflammatory cytokine IFN-γ. Similarly, recent research published by the Poultry Health Laboratory at the University of Arkansas have validated IgA as a reliable serum biomarker for assessing intestinal inflammation (Hernandez-Patlan et al., 2019). In the current investigation, serum IgA levels were substantially lower in all experimental groups compared to control HS chickens at 21 and 42 days of assessment, suggesting that EO modulates the inflammatory response of HS. Only two hours after adding HS into the experimental groups, a substantial rise in body temperature was noted, which lasted the duration of the trial. Nonetheless, it was notable that birds in groups 3 and 4, all of which included betaine, considerably reduced IgA circulation detected in the blood stream.

Heat stress is related with decreased feed intake and inflammation, both of which have a strong association with decreased bone mineralization and bone repair (Thomas, 2011; Shen et al., 2011; Ohlsson & Sjögren 2015). Control chickens in this study which were subjected to cyclic HS exhibited a substantial decrease in bone mineralization as measured by tibia break strength and total ash from the tibia, corroborating the findings of a prior study (Ruff et al., 2020). However, on days 21 and 42, groups 3 and 4 that got a combination of EO and betaine exhibited a substantial improvement in bone metrics.

Conclusions

Findings from this study suggest that supplementing and combining EO from Lippia origanoides, Rosmarinus officinalis, and either beetroot or natural betaine improves prowth performance, carcass component weights, intestinal permeability, antioxidant, and anti-inflammatory properties in broiler chickens subjected to cyclic HS. This study further revealed that, when compared to thermoneutral control broilers, heat stress lowered BW, feed intake, and bone strength through bone mineralization while increasing feed conversion, gut permeability, IFN-, and IgA levels. However, using EO and betaine strategically during a stressful time, such as heat stress, may assist to minimize the deleterious effects on broiler chickens.

Further studies are being conducted to validate the influence of EO supplementation on the aforementioned characteristics. Additionally, their bactericidal activity against Clostridium perfringens, are now being studied in a necrotic enteritis laboratory model.

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