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Effects of hawthorn (*Crataegus oxycantha*) fruit extract on tibia bone properties in broiler chickens subjected to heat stress

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ABSTRACT: The purpose of this study was to examine the effects on tibia morphology, biomechanics, and mineral levels of varying dosages of hawthorn (Crataegus oxyacantha) fruit extract (HFE) addition to experimentally heat-stressed broiler drinking water**.** A 2x3 factorial experimental design was used to randomly assign 300 one-day-old male broilers to six experimental groups. The experimental groups included three different HFE additions (0, 0.2, and 0.4 mL/L) and two different ambient temperatures (24 and 35 °C). Each experimental group consisted of five replicates, with 10 chicks (initial body weight 38±4 g) per replicate. The study results indicated that heat stress ~~(HS)~~ led to a decline in tibia dry weight, length, wet weight, volume, Seedor index, cortex thickness, shear force and shear stress, while concurrently resulting in an increase in dry matter percentage ($P<0.05$). Heat stress led to a decline in the levels of calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn) and potassium (K) ($P<0.05$). The addition of HFE in the drinking water of broiler chickens exposed to HS did not significantly affect the morphological and biomechanical properties of the tibia. However, tibial Ca levels increased with the addition of 0.2 mL/L HFE, while Mg and Zn levels were elevated at both 0.2 and 0.4 mL/L HFE concentrations compared to the control group ($P<0.05$). In contrast, Fe and Mn levels decreased with 0.2 mL/L HFE supplementation, and K levels were reduced at both supplementation levels compared to the control ($P<0.05$). The findings suggest that the addition of 0.2 mL/L HFE to the drinking water of heat-stressed broiler chickens may be an effective and recommended strategy to enhance bone quality characteristics.

Keyword: Broiler chickens; heat stress; hawthorn fruit extract; tibia bone.

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INTRODUCTION

The increasing global demand for animal-derived foods has significantly contributed to the intensification and industrialization of poultry production systems (Santos et al., 2022). In line with this demand, the broiler industry has implemented genetic selection programs aimed at maximizing growth rate and carcass yield, thereby markedly increasing meat production capacity (Cartoni Mancinelli et al., 2020). However, this genetic advancement has exceeded the physiological limits of modern broilers, giving rise to various health issues. In particular, the rapid attainment of high body weight leads to imbalances between the skeletal and circulatory systems, resulting in conditions such as pulmonary hypertension, sudden death syndrome, skeletal deformities, and bone fractures (Pritchard et al., 2020). The skeletal systems of these genetically selected broilers often fail to support the accelerated body mass adequately, with increased cortical bone porosity contributing to a heightened risk of deformities and fractures (Sanchez-Rodriguez et al., 2019). Additionally, the excessive body weight in fast-growing broilers restricts mobility, thereby reducing physical activity levels. This decrease in mechanical loading negatively affects bone mineralization and compromises skeletal development (Shim et al., 2012). Skeletal disorders not only impair animal welfare but also lead to considerable economic losses due to reduced carcass quality, poor feed conversion efficiency, and diminished growth performance (Yan et al., 2019). Lameness and restricted mobility impede access to feed and water, thereby lowering growth rates and contributing to early culling, increased mortality, and greater labor costs (Mohammed et al., 2021). Current evidence indicates that skeletal abnormalities cause annual losses amounting to billions of dollars in the poultry industry (Mohammed et al., 2021; Santos et al., 2022; Alharbi et al., 2024; Li et al., 2025).

Increasing temperature due to global warming and climate changes experienced all over the world is one of the important stress sources of the poultry sector (Malila et al., 2021; Attia et al., 2024; Yehia et al., 2025). Heat stress (HS) occurs in poultry when the ambient temperature exceeds 25°C, and the duration of exposure to high ambient temperature can last from several days to several weeks (Saracila et al., 2020; Hasan et al., 2022). Previous studies have reported that HS can cause undesirable physiological and biochemical effects in broiler chickens such as decreased weight gain and feed intake (Attia et al., 2017; Goo et al., 2019), carcass and meat quality (Liu et al., 2019; Chen et al., 2021), bone mass and quality (Jankowski et al., 2015; Hosseini-Vashan et al., 2016; Yan et al., 2019; Zhang et al., 2021; Rocchi et al., 2022; Marques-Carvalho et al., 2023), and suppression of the immune system (Hasan et al., 2022). High environmental temperature causes oxidative stress in poultry by increasing the concentrations of reactive oxygen species (ROS) and decreasing the efficiency of the antioxidant defence system (Mishra and Jha, 2019). Oxidative stress in poultry causes biological damage, health disorders, low welfare and low growth rates as well as significant economic losses (Tang et al., 2022; Karadağoğlu and Şahin, 2023). Oxidative stress refers to a state in which the intracellular redox equilibrium is disrupted due to an excessive accumulation of ROS (Snezhkina et al., 2019). Oxidative stress primarily arises from the abnormal activation of enzymes producing ROS such as superoxide anion (O_2^-), hydroxyl radical (OH•), and hydrogen peroxide (H_2O_2), inhibition of antioxidant enzymes, and/or a decrease in exogenous and endogenous antioxidant levels (Marcucci et al., 2023). Oxidative stress is implicated in the pathogenesis of bone diseases (Wauquier et al., 2009), including osteoporosis (Kimball et al., 2021; Iantomasi et al., 2023), bone tumor development (Magwere et al., 2008; Gajewska et al., 2022), diabetes-induced bone complications (Hamada et al., 2007; Sellmeyer et al., 2016; Chen et al., 2020) and joint inflammatory diseases (Wruck et al., 2011; Bolduc et al., 2019). In a state of oxidative stress, increased levels of ROS had opposite effects on osteoblast and osteoclast cells. ROS promote apoptosis by inhibiting osteoblast differentiation and perform this function by activating transcription factors known as Forkhead box O (FoxO) (Tian et al., 2017). In contrast to osteoblast differentiation, sustained increase of ROS inhibits osteoclast apoptosis and promotes osteoclastogenesis by increasing the expression of receptor activator of nuclear factor-kappa B ligand (RANKL) and activating extracellular signal-regulated kinase (ERK), nuclear factor kappa B (NF-κB), tumor necrosis factor (TNF) and interleukin 6 (Liang et al., 2024). On the other hand RANKL expression suppresses the transcriptional activity of FoxOs and promotes osteoclast differentiation and survival, accelerating bone resorption and preventing bone formation (Bartell et al., 2014). In order to protect against the negative effects of oxidative stress caused by HS in broiler chickens production, the modernist approach focuses on phytogenic dietary additives (Shakeri et al., 2019; Yan et al., 2019; Tekce et al.,

2020; Kaya, 2023a). Phytogenics contain a variety of active ingredients that have been demonstrated to possess antioxidant, antimicrobial, antifungal, antiviral, antilipidemic and digestive system stimulating effects. These dietary additives have attracted significant interest in modern animal nutrition due to their ability to enhance growth performance, feed conversion, carcass, immunity and bone health (Olgun, 2016; Cimrin et al., 2020; Huang et al., 2020; Tahami et al., 2021). Hawthorn fruit extract (HFE) is one of these phytogenic additives.

Hawthorn, which are common in Asia, Europe and North America, systematically belongs to the *Crataegus* genus of the *Rosaceae* family and are among the important medicinal plants that are frequently used in traditional and complementary medicine (Dahmer and Scott 2010; Gonz'alez-Jim'enez et al., 2018). They are a potential source of pectin, rich in flavonoids, anthocyanins (oligomeric procyanidins) and ascorbic acid (Liu et al., 2011), and contain many health beneficial substances, especially organic acids, fats and sugars (Liu et al., 2011; Cuevas-Bernardino et al., 2016). Hawthorn fruit contains approximately 9.94% pectin, 5.50% reducing sugars, 0.03% anthocyanins and 1.93% phenolic substances (Cuevas-Bernardino et al., 2016), as well as high amounts of various mineral substances, mainly calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg) and iron (Fe) (Özcan et al., 2005). It has been reported that administration of HFE (100 mg/kg once daily orogastrically) regulated serum total antioxidant status (TAS), total oxidant status (TOS) and OS index levels, decreased alveolar bone loss and showed inhibitory effect on periodontal inflammation and alveolar bone loss in experimental periodontitis induced wistar rats (Hatipoğlu et al., 2015). In a study investigating the effects of phytogenics on osteochondral tissue engineering by direct addition to carboxymethyl chitosan hydrogel scaffolds (Baysan et al., 2024), *Crataegus oxyacantha* extract increased collagen type I and type II formation and was found to be promising in osteochondral tissue regeneration. In the literature, medical and biochemical research results are frequently encountered emphasizing the antioxidant (Li et al., 2009; Dahmer and Scott, 2010; Jurikova et al., 2012), antimicrobial (Attard and Attard, 2019), hypolipidemic (Jurikova et al., 2012; Çetin, 2014) properties of HFE. On the other hand, while there are limited (Marcinčáková et al., 2011; Zhang et al., 2014; Ahmadipour et al., 2017; Song et al., 2018) studies investigating its effects on economically important farm animals, no research has been found examining its effects on the tibia bone in poultry.

Based on its antioxidant properties, HFE was hypothesized to contribute to the improvement of bone quality in animals under stress. Therefore, in this study, the effects of HFE (*Crataegus oxycantha*) addition to broiler chickens housed under HS on tibia bone morphological and biomechanical properties and tibia minerals are investigated.

MATERIALS AND METHODS

Animal care and housing

This study was conducted in accordance with the approval of the Local Ethics Committee for Animal Experiments of the Directorate of Veterinary Control Central Research Institute (Approval Date: 18.09.2020; Approval No: 2020/13). A total of 300 one-day old male broiler (Ross 308) chicks purchased from a commercial hatchery (Garanti Poultry Company, Bolu, Türkiye) were used in the experiment. The study was conducted in full compliance with animal welfare at Poultry Unit of Kelkit Organic Agriculture Research and Application Center of Gümüşhane University. The study lasted for a total of 42 days, including an initial 7-day acclimatization period and then 35-day fattening periods. After the acclimatization period, the chicks were weighed and randomly distributed in 30 wire pens (10 chicks/pen). The pens measured 121x110x108 cm and they had ~10 cm deep wood shavings on the floor. Each pen was equipped with hanging feeders and drinkers.

The poultry research house including of these pens had two identical rooms that can be separated with a door and were also heated by electric heaters. During the first 21 days of the experiment, rooms were not separated and standard brooding temperatures were applied to both rooms with temperature gradually decreased from 33°C to 24°C by the end of the third week of age. Chickens in each wire pens were randomly assigned to 6 experimental groups, 5 replicates of 10 chickens each in a 2 (temperature treatments) x 3 (dietary treatments) factorial arrangement from 21st to 42nd days of age. At the 21th days, the experiment rooms were separated from each other and 3 of 6 experimental groups were subjected to either thermoneutral temperature or HS treatments. Applied temperatures in the rooms were as follows. In thermoneutral temperature room (TN), chickens were kept at around 24°C for 24 hours between the 21st and 42nd days (Aviagen, 2009; Sahin et al., 2016). Relative humidity in this room ranged from 50% to

60% during the experiment. In heat stress temperature room (chickens were exposed to 34°C for 8 h/d (from 09:00 to 17:00 h) and then to 24°C for 16 h/d (from 17:00 to 09:00 h) between the 21st and 42nd days (Sahin et al., 2016; Malila et al., 2021; Rocchi et al., 2022). Relative humidity ranged from 70% to 80% from the 21th day until the end of the study. Temperature and humidity were monitored in each room at four locations using a temperature- humidity recording system. A fluorescent lighting schedule of 24 h light was used during the study with an average light intensity of 40 lux/m^2.

Diets

In the study, broiler starter (0-10 days), grower (11-24 days) and finisher feeds (25-42 days) were used, which were purchased from Abalıoğlu Agricultural Production Incorporated Company. Since HFE used in the experiment was given with drinking water, the chickens consumed the same feeds in the same periods.

In the study, in which the effects of 3 different extract levels were examined control (0), 0.2 and 0.4 ml of extract was added to a liter of water. HFE added to the drinking water of broiler chickens was obtained from a commercial company called Toroslar Group (Istanbul, Türkiye). By calculating the amount of extract that broilers/chickens housed in TN conditions would take with the water they would drink in 24 hours (Aviagen, 2009), it was ensured that all groups, except the control groups, received the same amounts of these additives. For this, the drinkers of all groups were collected for 1 hour every day at 17:00 and the animals were left without water. At the end of this period, the predetermined amount of HFE was dissolved in 100 mL of water and administered to the treatment groups, while the control groups received only 100 mL of plain drinking water. The solutions were provided to the chickens using one-liter drinkers. Following the completion of these, hanging chicken drinkers were put into use and the experiment was continued with fresh water (Kaya, 2019). Thus, it is ensured that the additives do not lose their bioactivity, and it is also prevented that the increased water consumption in the HS groups leads to more extract intake. Consumption of feed and additive-free drinking water was provided *ad libutum* throughout the experiment.

Analytical Procedures

Feed analysis

Crude nutrient analysis of the feeds used in the research Abalıoğlu Agricultural Production Incorporated Company were determined with the FOSS brand Near-infrared spectroscopy (NIRS) device in the laboratory and metabolic energy contents were calculated according to TSE (1991). The ingredients and chemical compositions of basal diet used in the experiment are given in the previous article by Kaya (2023b).

Determination of Tibia Bone Morphological and Biomechanical Properties

Hens that were selected completely at random (two chickens from each replicate, ten chickens per treatment group, totalling 60 chickens) were killed by cervical dislocation, and then the right tibias with some attached flesh were collected. One of the right tibia bones from each replication was used to determine the mineral content of the bone, while the other right tibia was used to measure the morphological and mechanical properties of the bone. The sample tibias were placed in a plastic container and stored at −82ºC until analysis. The bone samples were thawed at room temperature for 6 h in an air-conditioned room before the measurements began.

Bones were excised from all flesh and proximal cartilages were removed. Robusticity (RI) and Seedor index (SI) were calculated by measuring the bone length, weight and volume before the bones were fractured to determine the mechanical properties of the bone. The distance between the distal and proximal ends was recorded as bone length using a digital caliper (precision of 0.001 mm). While the wet weight of the bone was weighed on a digital balance sensitive to 0.001 g, the bone volume was measured with a beaker. The RI value was calculated according to the report of Reisenfeld (1972), and the SI value was calculated according to the report of Seedor et al. (1991). The mean diameter and wall thickness (cortex thickness) of the tibia was measured using digital callipers from two points on the central axis of the fractured tibia, which was used to determine the mechanical properties. The bone mechanical properties were determined from the load-deformation curve generated from a three-point bending test (ASAE Standard S459, 2001) using BESMAK Testing Instrument (Model BMT-100S; BESMAK, Ankara, Türkiye) and the Test Works 4 software package (version Litest X; BESMAK, Ankara, Türkiye). The cross-head speed was 5 mm/min. Shear tests on the tibia were performed using a double-shear block apparatus. A shear force was applied to a 15 mm (0.59 in) segment at the midpoint of the diaphysis. Fracture energy was subsequently

calculated by integrating these measurements with the corresponding shear force–deformation curve data, following the method of Wilson and Ruszler (1996). These tests allowed the evaluation of the ultimate shear force and shear stress for each bone. The cortex cross-sectional area was calculated according to Equation (1).

$$Cortex\ cross-sectional\ area = \pi \frac{\left(\frac{diameter\ of\ tibia}{2}\right)^2 - \left(\frac{cavity\ diameter\ of\ tibia}{2}\right)^2}{2} \quad (1)$$

The shear stress was calculated according to Equation (2).

$$Shear\ stress = \frac{shear\ force}{cortex\ cross-sectional\ area} \quad (2)$$

These biomechanical traits of bone were defined by Wilson and Ruszler (1996) and Armstrong et al. (2002). The fractured bones, in which the biomechanical traits were determined, were dried in an oven at 105°C for 24 hours and then weighed on a digital scale sensitive to 0.001 g and dry weight of the tibia bone was determined (Sultan et al., 2018). The dry matter ratio of tibia bone was calculated according to Equation (3). Then, these bones were burned in a muffle furnace at 600°C for 4.5 hours according to the procedures described by AOAC (1994) and weighed on a digital scale sensitive to 0.001 g, and the ash weight was determined (Sultan et al., 2018).

$$Dry\ matter\ (\%) = \frac{dry\ weight\ of\ tibia\ bone}{wet\ weight\ of\ tibia\ bone} \times 100 \quad (3)$$

The ash ratio of tibia bone was calculated according to Equation (4).

$$Ash\ of\ tibia\ bone\ (\%) = \frac{ash\ weight\ of\ tibia\ bone}{dry\ weight\ of\ tibia\ bone} \times 100 \quad (4)$$

Determination of tibia mineral contents

Tibia bones, which were thawed one day before the analysis, were cleaned of meat, fat and bone marrow, dried at 100 °C for 24 hours and ground to a size that could pass through a 0.5 mm sieve. 7 ml of nitric acid (HNO_3), 1 ml of hydrogen peroxide (H_2O_2) were added on 0.5 g tibia bone sample and burned in 2 different steps (1st step; 15 minutes at 200°C, TFM 120 W per pot, 2nd step; 15 minutes at 200°C, TFM 120 W per pot) in a microwave wet burning unit (Milestone, START D, Italy) resistant to 45 bar pressure. Then, the tibia bone mineral contents were determined using the Atomic Emission Spectrophotometer (ICP MS, Agilent Technologies 7700 Series, Japan) device (Inductively Coupled Plasma Mass Spectrophotometer) in the samples made up to 25 ml with distilled water (Mertens, 2005).

Statistical analysis

The data obtained from the experiments were evaluated using the 2x3 factorial statistical analysis model (General Linear Model procedure) with the help of SPSS 23.0 (IBM SPSS, Chicago, IL, USA) program. Duncan's multiple comparison test was used to compare the effects of the doses of additives, and HS.

RESULTS

Tibia bone morphological and biomechanical properties

The effects of different levels of HFE supplementation to the drinking water of broiler chickens exposed to HS on tibia dry weight (TDW), tibia dry matter (TDM), and tibia ash weight (TAW) and tibia ash percentages (TAP) are given in Table 1, and the effects on morphological and biomechanical properties are given in Table 2. HS decreased TDW ($P<0.05$) and increased TDM ($P<0.01$), but had no effect on TAW and TAP ($P>0.05$). Addition of HFE to the drinking water of stressed broiler chickens had no effect on TDW, TDM, TAW and TAP parameters ($P>0.05$). Heat stress caused a decrease in tibia bone length, wet weight, volume, SI ($P<0.01$) and cortex thickness, shear force and shear stress parameters ($P<0.05$), but had no effect on tibia bone RI, diameter, cortex cross-section area and fracture energy parameters ($P>0.05$). It was determined that the addition of 0.4 ml/L HFE to drinking water had no effect on other bone morphological and biomechanical properties ($P>0.05$) except for decreasing bone length compared to the control ($P<0.05$). In addition, the T*HFE interaction on these parameters was not significant ($P>0.05$).

Tibia bone minerals

The effects of different levels of HFE supplementation to the drinking water of broiler chickens subjected to HS on tibia bone mineral contents are given in Table 3. It was determined that housing under high environmental temperature did not affect tibia bone P and manganese (Mn) levels ($P>0.05$), but caused a decrease in tibia bone Ca ($P<0.05$), Mg, copper (Cu), Fe, zinc (Zn) and K levels ($P<0.01$). Only 0.2 mL/L HFE addition to drinking water increased tibia bone Ca content ($P<0.01$). The addition of 0.2 mL/L extract decreased ($P<0.01$) the Cu content of the tibia bone, but the addition of 0.4 ml/L extract

Table 1. Effect of hawthorn (*Crataegus oxycantha*) fruit extract supplementation in drinking water on tibia bone weight and ash of broiler reared under heat stress

		HFE (ml/L water)	TDW (g)	TDM (%)	TAW (g)	TAP (%)
T	TN	0	8.625 [ab]	47.0 [c]	3.454	40.0
		0.2	8.782 [a]	46.5 [c]	3.746	42.6
		0.4	8.372 [ab]	46.4 [c]	3.417	40.9
	HS	0	8.355 [ab]	47.7 [bc]	3.431	41.1
		0.2	7.843 [ab]	51.5 [a]	4.000	39.5
		0.4	7.721 [b]	50.0 [ab]	3.163	40.9
SEM			0.133	0.5	0.088	0.7
T	TN		8.593	46.6	3.539	41.2
	HS		7.973	49.7	3.231	40.5
HFE	0		8.490	47.3	3.443	40.6
	0.2		8.313	49.0	3.423	41.0
	0.4		8.046	48.2	3.290	40.9
p-values						
T			0.02	0.01	0.09	0.66
HFE			0.35	0.23	0.74	0.96
T*HFE			0.55	0.08	0.35	0.52

HFE, hawthorn fruit extract; T, temperature; TN, thermo-neutral; HS, heat stres; SEM, standart error of the mean; TDW, tibia dry weight; TDM, tibia dry matter; TAW, tibia ash weight; TAP, tibia ash percentages
[a-c.] Values within a column with different superscripts differ significantly (P<0.05) (Duncan's test)

increased it (P<0.01). Additionally, T*HFE interaction was found to be significant on bone Ca, Cu, Fe, Zn, Mn, K (P<0.01) and P (P<0.05) levels, but no interaction was observed between T*HFE for bone Mg level (P>0.05).

DISCUSSION-

Tibia bone morphological properties

In one of our parallel studies, Kaya (2023a) reported that HS negatively affected performance, serum malondialdehyde (MDA), immunoglobulin G (IgG) and corticosterone levels, and the parameters were improved with HFE supplementation. In the current study, the effects of HFE feeding on tibial bone characteristics of broilers exposed to high ambient temperature were investigated. RI and SI values are indicators of tibia bone density, and in contrast to SI, the lower the RI value, the denser the tibia bone (Abdelaziz, 2015; El-Faham et al., 2017). The relevant literature reports that HS does not affect the length, weight and density of the tibia bone (Zhang et al., 2021) and reduces the ash percentage of the tibia (Hosseini-Vashan et al., 2016; Rocchi et al., 2022).

In this study, the addition of different doses of HFE to the drinking water of broiler chickens exposed to HS had no effect on TDW, TDM, TAW and TAP, bone wet weight and volume, RI and SI parameters (P>0.05), but the length of the tibia bone was 0.4 mL/L. decreased in the level group (P<0.05). Since no study was found in the literature review examining the effects of adding different forms of hawthorn fruit to poultry diets on bone properties, a comparison was made with the effects of other herbal additives. While the results of the current study are compatible with some literature (Nkukwana et al., 2014; Cardinali et al., 2015; Mirakzehi et al., 2018; Pečjak et al., 2020; Mutlu et al., 2021) in terms of morphological features of the tibia bone, they are not compatible with some literature (Hosseini-Vashan et al., 2016; Huang et al., 2020; Tahami et al., 2021) information. It has been reported that the addition of 2% dried hops to chicken diets reduced bone length, similar to the current study, but did not affect tibia bone weight and ash weight (Kwiecień and Winiarska-Mieczan, 2009).

Tibia bone biomechanical properties

Rocchi et al. (2022) reported that cyclic HS decreased tibia shear force in broiler chickens and suggested

Table 2. Effect of hawthorn (*Crataegus oxycantha*) fruit extract supplementation in drinking water on the morphological and biomechanical properties of the tibia bone of broiler reared under heat stress

	HFE (ml/L water)	Length (mm)	Wet weight (g)	Volume (cm^3)	RI (cm/g)	SI (g/cm)	Diameter (mm)	Cortex thickness (mm)	Cortex cross-section area (mm^2)	Shear force (N)	Shear stress (N/mm^2)	Fracture energy (N.mm)
	0	104.9 ^{a}	18.37 ^{a}	14.00 ^{ab}	3.973	1.753 ^{a}	9.89	1.632	42.34	1982	46.8	2393
TN	0.2	105.8 ^{a}	18.88 ^{a}	14.58 ^{a}	3.977	1.783 ^{a}	9.94	1.644	42.86	1803	42.3	2664
	0.4	104.3 ^{ab}	18.07 ^{a}	14.22 ^{a}	3.979	1.732 ^{a}	9.75	1.548	39.92	1607	40.0	2715
T	0	104.6 ^{ab}	17.53 ^{a}	13.80 ^{ab}	4.031	1.676 ^{ab}	9.82	1.410	37.24	1275	34.1	1818
HS	0.2	101.5 ^{bc}	15.26 ^{ab}	11.86 ^{bc}	4.104	1.504 ^{b}	9.86	1.562	40.54	1141	28.2	1415
	0.4	100.4 ^{c}	15.46 ^{ab}	11.50 ^{c}	4.035	1.539 ^{b}	9.43	1.550	38.54	1155	30.0	2021
	SEM	0.5	0.35	0.34	0.022	0.029	0.10	0.022	0.78	117	2.7	280
T	TN	105.0	18.44	14.27	3.976	1.756	9.86	1.61	41.71	1797	43.0	2591
	HS	102.2	16.09	12.39	4.057	1.573	9.70	1.51	38.77	1190	30.8	1751
	0	104.7 ^{a}	17.95	13.90	4.002	1.715	9.85	1.521	39.79	1629	40.4	2106
HFE	0.2	103.6 ^{ab}	17.08	13.22	4.040	1.504	9.90	1.603	41.70	1472	35.3	2040
	0.4	102.4 ^{b}	16.76	12.86	4.007	1.539	9.59	1.549	39.23	1381	35.0	2368
p-values												
T		0.01	0.01	0.01	0.08	0.01	0.48	0.03	0.06	0.02	0.03	0.16
HFE		0.05	0.18	0.35	0.75	0.34	0.47	0.28	0.39	0.65	0.65	0.89
T*HFE		0.13	0.11	0.15	0.76	0.24	0.87	0.10	0.58	0.88	0.95	0.88

HFE, hawthorn fruit extract; T, temperature; TN, thermo-neutral; HS, heat stres; SEM, standart error of the mean; RI, Robusticity index; SI, Seedor index

$^{a-c,}$ Values within a column with different superscripts differ significantly ($P<0.05$) (Duncan's test).

Table 3. Effect of hawthorn (*Crataegus oxycantha*) fruit extract supplementation in drinking water on tibia bone mineral elements of broiler reared under heat stress

		HFE (ml/L water)	Ca (ppm)	P (ppm)	Mg (ppm)	Cu (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)	K (ppm)
T	TN	0	159567 [b]	68587 [ab]	3840 [b]	16.8 [c]	133 [a]	107 [c]	3.39 [a]	3890 [a]
		0.2	184834 [a]	67839 [ab]	3977 [a]	19.4 [b]	101 [c]	149 [a]	1.91 [c]	3106 [c]
		0.4	146299 [c]	63750 [ab]	4013 [a]	20.6 [a]	118 [b]	75 [e]	2.79 [b]	3694 [b]
	HS	0	140662 [c]	61186 [b]	3458 [d]	14.7 [d]	76 [e]	67 [f]	2.47 [b]	2968 [d]
		0.2	149666 [bc]	68875 [ab]	3675 [c]	10.8 [e]	65 [f]	83 [d]	2.08 [c]	2476 [e]
		0.4	158891 [b]	69747 [a]	3772 [bc]	14.5 [d]	86 [d]	120 [b]	3.25 [a]	2427 [e]
		SEM	2784	1116	36	0.6	4	5	0.10	94
T		TN	163567	66725	3943	18.9	117	110	2.70	3563
		HS	149740	66603	3635	13.3	76	90	2.60	2623
HFE		0	150115 [b]	64887	3649 [b]	15.8 [b]	104 [a]	87 [c]	2.93 [a]	3425 [a]
		0.2	167251 [a]	68357	3826 [a]	15.1 [c]	83 [b]	116 [a]	2.00 [b]	2791 [c]
		0.4	152595 [b]	66749	3893 [a]	17.5 [a]	102 [a]	98 [b]	3.02 [a]	3061 [b]
p-values										
T			0.05	0.95	0.01	0.01	0.01	0.01	0.38	0.01
HFE			0.01	0.42	0.01	0.01	0.01	0.01	0.01	0.01
T*HFE			0.01	0.05	0.29	0.01	0.01	0.01	0.01	0.01

HFE, hawthorn fruit extract; T, temperature; TN, thermo-neutral; HS, heat stres; SEM, standart error of the mean; Ca, Calcium; P, Phosphorus; Mg, magnesium; Cu, Copper; Fe, Iron; Zn, Zinc; Mn, Manganese; K, Potassium

[a-f.] Values within a column with different superscripts differ significantly (P<0.05) (Duncan's test)

that the increase in intestinal permeability caused by temperature stress was associated with decreased bone strength. The results of the present study are consistent with other studies (Hosseini-Vashan et al., 2016; Zhang et al., 2021; Rocchi et al., 2022) showing that HS affects bone mass and strength and also impairs bone healing and regeneration. The addition of HFE to the diets of broilers exposed to HS had no effect on tibia biomechanical properties (P>0.05). Bone is a dynamic tissue affected by factors such as nutrition, stress and physical activity (Olgun et al., 2022). It was observed that the current study results were compatible with some studies (Nkukwana et al., 2014; Cardinali et al., 2015; Olgun, 2016; Leskovec et al., 2017; Mirakzehi et al., 2018; Mutlu et al., 2021) examining the effects of different phytogenic feed additives added to the diets of experimental animals housed under TN environmental conditions on bone biomechanical properties, but were partially compatible with some other studies (Kwiecień et al., 2014; Hosseini et al., 2016). In this study, the fact that the addition of 0.2 mL/L HFE to the diets of broiler chickens housed under HS increased bone diameter, cortex thickness and cortex cross-section area was considered important in terms of showing that the addition of HFE has positive effects on bone strength.

Tibia bone minerals

The current study findings, which indicated that HS application did not affect tibia bone P and Mn but decreased Ca, Mg, Cu, Fe, Zn and K, are consistent with existing literature (Hosseini-Vashan et al., 2016; Yan et al., 2019). However, in addition to negatively affecting bone mineral levels, HS application also negatively affected some morphological (weight, length, volume) and biomechanical (bone cortex thickness, shear force and shear stress) properties of the bone (Table 1 and Table 2). It is thought that the decrease in walking activity due to rapid growth, increased corticosterone hormone secretion level due to stress and inadequate feed consumption (Kaya, 2023a) cause these adverse effects. Heat stress is one of the factors causing bone damage (Zhang et al., 2021; Rocchi et al., 2022), and stimulates the activity of the hypothalamic-pituitary-adrenal axis

and increases serum corticosterone hormone levels (Elnesr et al., 2019; Kaya, 2023a). High levels of glucocorticoid concentration negatively affect bone mass by inhibiting osteoblastogenesis, increasing osteoblast and osteocyte apoptosis, and promoting the presence of osteoclast cells (Henneicke et al., 2014; Yan et al., 2019). Increased body temperature with high environmental temperature contributes to skeletal damage by increasing the ROS level in the mitochondria and causing OS (Huang et al., 2015). It has been reported that HS in broiler chickens disrupts intestinal function and reduces feed consumption, nutrient absorption and live weight gain (Jankowski et al., 2015). The decrease in feed consumption due to the effect of high temperature leads to bone loss by reducing Ca consumption and absorption in turkeys (Jankowski et al., 2015) and broilers (Hosseini-Vashan et al., 2016).

According to the results of the current study, the tibia bone Ca level of broiler chickens raised under HS increased with the addition of 0.2 ml/L HFE, the Cu level increased with the addition of 0.4 ml/L HFE, and the Mg and Zn levels increased ($P<0.01$) at both HFE supplementation levels. Cu, Fe and Mn levels decreased with the addition of 0.2 ml/L HFE, and K level decreased at both HFE addition levels ($P<0.01$). It was notable that different HFE levels affected bone Cu content in two different ways compared to the control. Although the tibia P level tended to increase without reaching statistical significance, it was not statistically affected by the addition of HFE ($P>0.05$). Bone mineralization is an important indicator of the health status of the organism, as well as the effectiveness and quality of nutritional procedures (Kwiecień and Winiarska-Mieczan, 2009). The results of the current study, in which it was determined that HFE additions to the drinking water of broiler chickens treated with HS were effective on bone minerals, are compatible with some studies (Nkukwana et al., 2014; Hosseini-Vashan et al., 2016; Mirakzehi et al. 2018; Tahami et al., 2021) examining the effects of adding different phytogenic additives to poultry diets on bone minerals, but are not compatible with some other studies (Olgun, 2016; Hosseini et al., 2016; Huang et al., 2020; Pečjak et al., 2020). When the current study is compared with studies under TN conditions, it can be said that phytobiotics added to the diet of broiler chickens under high ambient temperatures are more effective in increasing intestinal mineral and nutrient absorption (Yan et al., 2019). According to Martínez et al., (2020) ideal intestinal pH conditions are needed for the insolubility or instability of the Ca mineral. Previous studies have reported that phytobiotics improve intestinal integrity (Tekce et al., 2020; Yılmaz, 2022), regulate intestinal pH, and as a result, increase the absorption and bioavailability of minerals such as Ca and P for bone mineralization (Savón et al. 2007; Xu et al., 2011; Martínez et al., 2020).

CONCLUSIONS

In conclusion, the supplementation of varying levels of HFE in the diets of broiler chickens exposed to high ambient temperatures did not significantly affect certain morphological and biomechanical properties of the tibia bone; however, it led to increased levels of Ca, Mg, Zn and Cu in the tibia. These findings suggest that adding 0.2 mL/L HFE to the drinking water of heat-stressed broiler chickens may have beneficial effects in mitigating stress-induced impacts and enhancing bone quality characteristics.

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1. https://arccjournals.com/journal/indian-journal-of-animal-research/BF-1652
2. https://arccjournals.com/journal/indian-journal-of-animal-research/BF-1657

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this study.

Authors' Contribution Statements: All authors contributed to the conception and design of the study. Material preparation and data collection were performed by H. Kaya. All analyses, except for statistical evaluations, were conducted by H. Kaya, while statistical analyses were carried out by M. Karaalp. The first draft of the manuscript was written by H. Kaya and subsequently reviewed and edited by M. Karaalp. All authors provided feedback on the final version of the manuscript and approved it for submission.

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