



The Effect of Organic Hawthorn (*Crataegus tanacetifolia*) Fruit Vinegar Supplement on Growth Performance, Carcass Characteristics and Some Serum Parameters in Broiler Chickens Subjected to Cyclic Heat Stress^[1]

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ABSTRACT

Background: This study, it was aimed to determine the effects of adding organic hawthorn (*Crataegus tanacetifolia*) fruit vinegar (HFV) in different doses to the drinking water of broiler chickens reared under cyclic heat stress (CHS) on performance, carcass characteristics and some serum parameters.

Methods: In the study, 300 one-day-old broiler chickens (Ross 308), after a 1-week acclimation period, were tested in two different (24 and 35°C) ambient temperatures and three different HFV levels (0, 2 and 4 ml/L) according to the 2×3 factorial experimental design. Chicks were randomly distributed to 6 experimental groups with 5 replications, with 10 animals in each replication. The addition of organic HFV to the drinking water of broiler chickens subjected to CHS didn't affect growth performance, carcass characteristics and visceral weight ($P>0.05$). Addition of HFV increased serum FFA level, decreased serum MDA, HDL and TAG levels ($p<0.05$) and did not affect other serum parameters ($P>0.05$) in CHS-treated groups.

Result: As a result, it was concluded that the addition of HFV to the drinking water of broiler chickens exposed to cyclic heat stress did not have any negative effect on growth performance, carcass characteristics and visceral weights, but it could be beneficial in preventing lipid peroxidation.

Key words: Broiler chickens, Carcass characteristics, Cyclic heat stress, Hawthorn fruit vinegar, Performance, Serum biochemistry.

INTRODUCTION

Heat stress (HS) is an important stress factor that causes health problems ranging from growth and developmental retardation, metabolic problems to death in poultry due to its immunosuppressive effect (Bayraktar *et al.*, 2021). It has been reported that HS induces growth retardation, weakening of the immune system and reduced resistance to diseases in broiler chickens production, leading to decreases in product quantity and quality (Liu *et al.*, 2019). In addition, it is important to reduce and prevent the negative effects of HS, which causes serious economic problems in the poultry industry (Bayraktar *et al.*, 2023).

There is increasing interest in hawthorn fruit vinegar (HFV) because of the antioxidative, antimicrobial anti-inflammatory, cardiovascular-protective and hypolipidemia effects of bioactive substances such as acetic acid, gallic acid, catechin, epicatechin and chlorogenic acid in its nature (Özdemir *et al.*, 2022). This study aimed to investigate the effects of adding 0, 2 and 4 ml/L organic HFV to broiler drinking water grown under cyclical heat stress (CHS) on growth performance, carcass characteristics, visceral weights and some serum parameters.

MATERIALS AND METHODS

This study was guided pursuant to the approval (dated 18.09.2020 and numbered 2020/13) of the Local Ethics

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Animals and experimental design

In the study, 300 1-day-old male broiler chickens (Ross 308) were used as animal material. After a 7-day acclimation period, chicks were randomly placed in two identical rooms at two different ambient temperatures [24°C; thermoneutral (TN) and 35°C; cyclic heat stress (CHS)] and 3 different HFV levels (0, 2 and 4 ml/L water) in accordance with a 2×3

trial design. The study was carried out with 5 replications and 10 chickens were used in each replication. The broiler chick were placed in a total of 30 pens, 15 in each room, which were 121×110×108 cm in size with sawdust litter, containing a hanging manger, drinker and automatic heaters. The temperature of the trial rooms, which were kept at 33°C for the first 7 days, was gradually reduced to 24°C until the 21st day. After day 21, one house was kept in TN conditions (approximately 24°C) but the other house (CHS) was kept at 34°C for 8 h per day (09:00-17:00) and then 24°C for 16 h/h to (from 17:00 to 09:00) and during the study, a 24-h lighting program was applied to the chickens and the intensity was around 40 lux/m² (Sarica *et al.*, 2015).

Feed

The feeds and organic HFV used in the study were obtained from a private company. Broiler starter feed was used between days 0-11, grower feed between days 11-24, finisher feed between days 25-42 (Table 1). Since HFV was given with drinking water, the feeds used in the study were the same for all animals. Every day at 16:00, the drinkers of all groups were turned off for 1 hour and the animals were dehydrated. At the end of this period, each treatment group was given 100 ml of pure water through a 1 liter drinker and 100 ml of pure water was added to the experimental groups until the water was consumed by adding HFV to the experimental groups and then the hanging chicken drinkers were activated and the experiment was continued with fresh water.

Determination of performance parameters

Weight gain (WG) was determined by individually weighing all animals on days 7, 14, 21, 35 and 42 of the study. The amount of feed consumed in each compartment was taken into account in calculating the FI and FCR value. The FCR value was expressed as the amount of feed consumed per unit body weight gain (g/g). The chickens that died during the experiment were recorded daily and taken into account in the calculation of the FCR value. The chickens that died during the experiment were recorded and were taken into account when calculating the FCR.

Determination of carcass characteristics and internal organ weights

On the 42nd day of the study, depending on complete chance, 10 chickens from each group, 2 animals from each replication, 60 chickens in total, were selected from the chickens that were starved from the evening and slaughtering was performed by the cervical dislocation (Tekce *et al.*, 2020). Hot carcass weights were obtained by weighing at room temperature approximately 45 minutes after slaughter, while cold carcass weights were obtained by weighing after 24 hours at 4°C (Sabaw and Muhammed, 2021). All weights were obtained by weighing on a balance (SHIMADZU BL-3200H, Germany) sensitive to 0.001 g. Carcass characteristics and internal organ weights were measured as a percentage of body weight.

Feed analysis

A raw nutrient analysis of the feeds used in the research was conducted using a Near-infrared spectroscopy device. The nutrient contents and chemical composition of the basal feed used in the experiment are given in Table 1.

Analyzes of hawthorn fruit vinegar

The total phenolic compound quantities were determined using a Folin-Ciocalteu reagent and the method described by Singleton *et al.* (1999), with slight modifications (Gülçin *et al.* 2002). The total phenolic content of HFV was determined as 1551.29 mg gallic acid equivalent (GAE)/L. The 2, 2'-Azino-bis (3-ethylbenzotriazolin-6-sulfonik asit) (ABTS•+) scavenging activity was determined using the method described by Köksal *et al.* (2009). It was determined that the ABTS scavenging activity of HFV was 11.075 (IC₅₀, µg/mL). Phenolic component and organic acid profile analyzes of HFV were determined using the high-performance liquid chromatography (HPLC) method (Coklar and Akbulut, 2017). The phenolic compound and organic acid profiles is presented in Table 2.

Biochemical analysis

Serum total antioxidant capacity was determined by colorimetric method (Erel, 2004); serum total oxidant capacity (Erel, 2005) and serum malondialdehyde (MDA) (Ohkawa *et al.*, 1979) was determined by spectrophotometric method; serum IgG (Li *et al.*, 2000) and corticosterone (Sahin *et al.*, 2003) concentrations was determined by the ELISA method. Serum-free fatty acid (FFA) and triacylglyceride (TAG) levels were determined by HPLC method (Sherma, 2003). Serum levels of glucose, minerals, alanine aminotransaminase, aspartate aminotransferase, alkaline phosphatase, total cholesterol, low-density lipoprotein, high-density lipoprotein, glucose parameters were measured with a fully automatic biochemistry device Cobas 8000 (Roche, Germany).

Statistical analysis

The data obtained from the trials were evaluated using the 2×3 factorial statistical analysis model (General Linear Model procedure) with the help of SPSS 23.0 program. Duncan multiple comparison test was used to compare the effects of the doses of the additives and the T-test (Independent Samples T Test) was used to compare the effects of heat stress.

RESULTS AND DISCUSSION

Growth performance

The effects of adding different doses of organic HFV to broiler chickens water reared under CHS on performance parameters (FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio) are shown in Table 3. It was determined that CHS decreased FI ($P<0.05$) but had no effect on WG and FCR ($P>0.05$). According to group averages, HFV additions to drinking water did not affect WG

Table 1: Composition of the experimental basal diet.

Component g/kg	Starter diet (1-10. days)	Grower diet (11-24. days)	Finisher diet (25-42. days)
Maize	480.0	505.0	545.0
Wheat feed flour	65.0	40.0	40.0
Soybean meal (48%)	375.0	285.0	230.0
DDGS-dried distiller's grains with solubles)	-	75.0	75.0
Soybean oil	40.0	55.0	65.0
Corn gluten meal	10.0	14.0	20.0
Limestone	7.5	7.0	7.0
Dicalcium phosphate	11.0	9.5	9.0
Threonine	1.5	1.0	1.0
DL-Methionine	3.2	2.5	2.5
L-Lysine	2.7	2.5	2.5
Premix ¹	1.0	1.0	1.0
Enzyme ²	-	1.0	1.0
Salt	2.6	1.0	1.0
Anticoccidial	0.5	0.5	-
Total	1000	1000	1000
Analysed composition (%dry matter, DM) (per kg)			
ME, kcal/kg*	3009.8	3102.1	3209.5
Dry matter (g)	889.7	892.5	893.9
Crude protein (g)	231.3	210.3	191.70
Ether extract (g)	65.33	88.94	99.92
Crude ash (g)	54.89	53.21	52.36
Starch (g)	372.88	373.39	373.65
Crude fiber (g)	34.66	37.80	32.48
Sugar (g)	43.30	37.10	33.81

¹Provides in kg of diet: Vitamin A (retinol) 9.000.000 IU, vitamin D3 (cholecalciferol) 4.000.000 IU, vitamin E (α tocopheryl acetate) 50.000 mg, vitamin K3 (menadione) 2.200 mg, vitamin B1 (thiamine) 2.200 mg, vitamin B2 (riboflavin) 5.000 mg, vitamin B3 (niasinamid) 47.500 mg, vitamin B5 (Ca-D-pantotenat) 16.500 mg, vitamin B6 (pyridoxine) 2.200 mg, vitamin B9 (folic acid) 1.250 mg, vitamin B12 (cyanocobalamin) 14 mg, vitamin H (D-biotin) 135 mg, Mn (manganese oxide) 105.000 mg, Fe (iron carbonate) 20.000 mg, Zn (zinc oxide) 82.000 mg, Cu (copper sulphate) 16.000 mg, I (calcium iodate), 1.200 mg, Se (sodium selenite) 300 mg.

²6- phytase;300 OTU/kg, 1-4 beta xylanase 3.000 EPU/kg.

*Calculated.

Table 2: Phenolic compound and organic acid profiles of hawthorn fruit vinegar.

Phenolics			Organic acids		
Parameters	Value		Parameters	Value	
	mg/L	%		mg/L	%
Gallic acid	104.12	10.6	Formic acid	1873.18	11.8
Catechin	18.24	1.9	Lactic acid	1314.94	8.2
Gentisic acid	308.44	31.6	Acetic acid	10776.21	67.7
Rutin	123.93	12.6	Fumaric acid	214.83	1.3
Ferulic acid	78.38	8.0	Succinic acid	1745.22	11.0
Naringin	24.57	2.5	Total	15924.38	100
Neohesperidin	83.71	8.6			
Coumarin	25.60	2.6			
Resveratrol	36.14	3.7			
Quercetin	54.77	5.6			
<i>t</i> -Cinnamic acid	82.76	8.5			
Hesperidin	29.77	3.1			
Flavone	6.85	0.7			
Total	977.28	100			

and FCR ($P>0.05$), FI decreased with the addition of 4 ml/L HFV ($P<0.05$), but HFV additions did not affect performance parameters in stressed groups ($P>0.05$). Acetic acid increases the feeling of satiety by slowing the emptying of the stomach (Hlebowicz *et al.*, 2008). It is thought that

acetic acid in the HFV content used in our study may cause a decrease in FI depending on the concentration (Table 2). While it is compatible with the results of our current study (Karaalp *et al.*, 2018; Goharizi *et al.*, 2020; Hanchai *et al.*, 2021; Rattanawut *et al.*, 2021), it differs

Table 3: Effect of hawthorn (*Crataegus tanacetifolia*) fruit vinegar supplementation in drinking water on performance of broiler chickens reared under cyclic heat stress.

Treatment	HFV, (ml/L water)	FI (g)	BWG (g)	FCR (g/g)
TN	0	4349	3062	1.420
T	2	4243	2967	1.433
	4	4195	2942	1.426
HS	0	4254	2931	1.452
	2	4117	2919	1.411
	4	4088	2945	1.388
	SEM	27	16	0.008
T	TN	4262	2990	1.426
	HS	4153	2932	1.417
HFV	0	4302 ^a	2997	1.436
	2	4180 ^{ab}	2943	1.422
	4	4142 ^b	2944	1.407
p-values				
T		0.04	0.06	0.54
HFV		0.04	0.25	0.31
T*HFV		0.97	0.18	0.17

HFV: Hawthorn fruit vinegar; T: Temperature; TN: Thermo-neutral; HS: Heat stres; SEM: Standart error of the mean; FI: Feed intake; BWG: Body weight gain; FCR: Feed conversion ratio (g FI/g WG).

^{a-b}. Values within a column with different superscripts differ significantly ($P<0.05$).

Table 4: Effect of hawthorn (*Crataegus tanacetifolia*) fruit vinegar supplementation in drinking water on carcass characteristics and visceral weights (% Body weight) of broiler chickens reared under cyclic heat stress.

Treatment	HFV (ml/L water)	Hot dressing	Cold dressing	Thigh dressing	Breast dressing	Heart	Spleen	Bursa fabricus	Abdominal fat
TN	0	66.6	66.0	25.8	21.0	0.496	0.112	0.142	1.58
	2	67.6	67.4	28.0	22.2	0.512	0.086	0.142	1.24
T	4	67.2	66.4	27.0	22.2	0.568	0.102	0.142	1.22
	0	68.6	67.8	27.4	22.0	0.540	0.086	0.140	1.54
HS	2	69.0	68.0	27.0	22.4	0.510	0.084	0.140	1.30
	4	67.2	66.0	26.0	22.6	0.556	0.086	0.136	1.22
	SEM	0.29	0.3	0.3	0.2	0.013	0.003	0.004	0.04
T	TN	67.1	66.6	26.9	21.8	0.525	0.100	0.142	1.35
	HS	68.3	67.3	26.8	22.3	0.535	0.085	0.139	1.35
HFV	0	67.6	66.9 ^{ab}	26.6	21.5	0.518	0.099 ^a	0.141	1.56 ^a
	2	68.3	67.7 ^a	27.5	22.3	0.511	0.085 ^b	0.141	1.27 ^b
	4	67.2	66.2 ^b	26.9	22.4	0.562	0.094 ^{ab}	0.139	1.22 ^b
p-values									
T		0.05	0.22	0.79	0.26	0.70	0.01	0.73	0.92
HFV		0.25	0.05	0.23	0.24	0.23	0.05	0.98	0.01
T*HFV		0.31	0.26	0.07	0.76	0.64	0.17	0.98	0.82

HFV: Hawthorn fruit vinegar; T: Temperature; TN: Thermo-neutral; HS: Heat stres; SEM: Standart error of the mean.

^{a-b}. Values within a column with different superscripts differ significantly ($P<0.05$).

(Omid *et al.*, 2020; Al-Shammari and Batkowska, 2021; Sittiya *et al.* 2021). We foresee that the reason for this situation is due to the difference in the material and method used in the research.

Carcass characteristics and visceral weights

The effects of adding different doses of organic HFV to the drinking water of broiler chickens raised under CHS on visceral weight and carcass quality are presented in Table 4.

Table 5: Effect of hawthorn (*Crataegus tanacetifolia*) fruit vinegar supplementation in drinking water on serum parameters of broiler chickens reared under cyclic heat stress.

Treatment	HFV (ml/L water)	TAS (μmol/L)	TOS (μmol/L)	MDA (mmol/L)	IgG (g/L)	Cort (nmol/L)	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	AST (U/L)	ALT (U/L)
TN	0	756	2.92	5.01 ^c	1.37	39.7	7.31	4.39	2.07 ^{ab}	179	6.57
	2	723	3.27	4.97 ^c	1.71	46.3	7.44	4.30	1.96 ^d	167	6.36
	4	695	3.46	4.98 ^c	1.81	48.0	7.35	4.43	2.00 ^{cd}	164	6.53
HS	0	680	3.26	7.75 ^a	1.98	54.5	7.40	4.57	2.06 ^{abc}	178	6.16
	2	755	2.92	6.55 ^b	2.34	66.3	8.04	4.84	2.10 ^a	173	6.34
	4	693	3.34	6.09 ^b	2.18	67.0	7.59	4.56	2.02 ^{bcd}	189	6.34
T	SEM	11	0.07	0.21	0.06	2.1	0.09	0.06	0.01	5	0.05
	TN	725	3.22	4.98	1.63	44.6	7.37	4.37	2.01	170	6.48
	HS	709	3.17	6.80	2.17	62.6	7.68	4.66	2.05	179	6.28
HFV	0	718	3.09	6.38 ^a	1.67 ^b	47.1 ^b	7.35	4.48	2.07 ^a	178	6.36
	2	739	4.00	5.76 ^b	2.03 ^a	56.3 ^a	7.74	4.57	2.03 ^{ab}	170	6.35
	4	694	3.40	5.53 ^b	2.00 ^a	57.5 ^a	7.47	4.49	2.01 ^b	176	6.43
p-values											
T		0.44	0.77	0.01	0.01	0.01	0.08	0.01	0.01	0.40	0.03
HFV		0.20	0.13	0.01	0.01	0.01	0.19	0.72	0.03	0.81	0.69
T*HFV		0.10	0.13	0.01	0.18	0.56	0.48	0.23	0.01	0.67	0.20

HFV: Hawthorn fruit vinegar; T: Temperature; TN: Thermo-neutral; HS: Heat stress; SEM: Standard error of the mean; TAS: Total antioxidant of serum; TOS: Total oxidant of serum; MDA: Malondialdehyde; IgG: Immunoglobulin G; CORT: Corticosterone; Ca: Calcium; P: Phosphorus; Mg, Magnesium; AST: Aspartate aminotransferase; ALT, Alanine aminotransferase.

^{a-d}. Values within a column with different superscripts differ significantly (P<0.05).

Table 6: Effect of hawthorn (*Crataegus tanacetifolia*) fruit vinegar supplementation in drinking water on serum parameters of broiler chickens reared under cyclic heat stress.

Treatment	HFV (ml/L water)	ALP (U/L)	FFA (%)	TAG (%)	T-Chol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	GLU (mg/dl)	T-PRO (g/dl)	ALB (g/dl)	GLB (g/dl)
TN	0	71.1	17.71 ^a	39.84 ^a	181	71.0 ^a	49.1 ^d	159	4.89	1.624	3.27
	2	67.1	9.21 ^c	32.81 ^b	165	60.6 ^b	55.8 ^c	177	5.03	1.676	3.35
	4	91.1	8.46 ^{cd}	30.12 ^{bc}	175	58.7 ^b	57.7 ^c	170	4.92	1.648	3.27
HS	0	73.7	7.28 ^d	27.56 ^{cd}	219	47.4 ^c	76.0 ^a	129	4.72	1.260	3.25
	2	67.8	11.81 ^b	26.14 ^d	238	45.6 ^c	70.7 ^b	165	5.25	1.782	3.47
	4	76.6	10.64 ^b	24.72 ^d	241	45.9 ^c	73.0 ^{ab}	139	4.89	1.594	3.30
T	SEM	4.3	0.83	1.27	6	1.8	1.9	4	0.07	0.059	0.06
	TN	76.4	11.79	34.26	174	63.4	54.2	169	4.95	1.649	3.30
	HS	72.7	9.91	26.14	233	46.3	73.2	144	4.95	1.545	3.34
HFV	0	72.4	12.49 ^a	33.70 ^a	200	59.2 ^a	62.5 ^b	144 ^b	4.81	1.442	3.26
	2	67.4	10.51 ^b	29.48 ^b	202	53.1 ^b	63.3 ^{ab}	171 ^a	5.14	1.729	3.41
	4	83.8	9.55 ^c	27.42 ^b	208	52.3 ^b	65.3 ^a	154 ^b	4.90	1.621	3.28
p-values											
T		0.68	0.01	0.01	0.01	0.01	0.01	0.01	0.95	0.36	0.77
HFV		0.32	0.01	0.01	0.57	0.01	0.04	0.01	0.16	0.13	0.65
T*HFV		0.69	0.01	0.02	0.11	0.01	0.01	0.16	0.52	0.23	0.93

HFV: Hawthorn fruit vinegar; T: Temperature; TN: Thermo-neutral; HS: Heat stress; SEM: Standard error of the mean; ALP: Alkaline phosphatase; FFA: Free fatty acid; TAG: Triacylglycerol; T-CHOL: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; GLU: Glucose; T-PRO: Total protein; ALB: Albumin; GLB: Globulin.

^{a-d}. Values within a column with different superscripts differ significantly (P<0.05).

According to our study results, it was determined that HFV supplementation had no statistical effect on carcass characteristics and visceral weights in stressed groups ($P>0.05$). The results of the present study are consistent with those reporting no change in carcass characteristics and visceral weights with the addition of vinegar to the diet of broiler chickens reared under thermoneutral conditions (Jahantigh *et al.*, 2021; Rattanawut *et al.*, 2021; Adeleye *et al.*, 2021), but contrast with those reported by Fu *et al.* (2013), Awaad *et al.* (2018) and Sittiya *et al.* (2021). As the reason for this situation, it was concluded that HFV is shaped depending on the phenolic profile and organic acid content (Table 2), the route of administration, dose, duration and material method difference.

Serum parameters

The effects of adding different doses of organic HFV to the drinking water of broiler chickens raised under CHS on some serum parameters are given in Table 5 and Table 6. Our study results show that the addition of HFV to the drinking water of broilers exposed to heat stress increases serum FFA levels but decreases serum MDA, TAG and HDL levels ($P<0.05$) and did not affect other serum parameters ($P>0.05$). It is thought that the decrease in serum MDA level observed in stressed groups may be related to the antioxidant activity ($11.075 \text{ IC}_{50} \mu\text{g/ml}$) and total phenolic substance (1551.29 mg GAE/L) levels in the structure of HFV. The results of our study are compatible with some study results (Hayajneh *et al.*, 2018; Awaad *et al.*, 2018; Hayajneh, 2019; Goharizi *et al.*, 2020; Yan *et al.*, 2020; Al-Shammari and Batkowska, 2021), but they differ with some study results (Kamal and Ragaa, 2014; El-Sahn *et al.*, 2021). As the reason for the difference in the study results, we predict that the HFV used in practice is shaped depending on the phenolic profile and organic acid content (Table 2), the route of administration, the dose and the experimental protocol.

CONCLUSION

As a result, while no negative effects were determined on the performance values, carcass and visceral weights of the addition of HFV to broiler drinking water grown under heat stress, it was concluded that it may contribute to the prevention of lipid peroxidation and it would be beneficial to examine different doses and administration routes.

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Conflict of interest

The author declare no conflict of interest.

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