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The Effects of Hawthorn (*Crataegus oxyacantha*) Fruit Extract on Performance, Carcass and Some Serum Parameters of Broilers Reared Under Heat-Stress Conditions[2]

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ABSTRACT

Background: The present experiment was conducted to investigate the effect of heat stress (HS) and hawthorn (*Crataegus oxyacantha*) fruit extract (HFE) on performance, carcass characteristics and serum parameters in broiler chickens.

Methods: Experimental treatments included two different ambient temperatures (24°C: thermoneutral conditions, or 35°C: HS conditions) and three different HFE (0, 0.2 and 0.4 ml/L) in a 2×3 factorial arrangement. A total of 300 1-day-old broiler chicks were randomly allotted 1 of 6 treatments with five replicates. A total of 300 1-day old broiler chicks were randomly allocated to 6 groups with five replications of 10 birds each. HFE supplementation had no effect on growth performance, carcass characteristics and visceral weight in stressed groups (P>0.05). It was also determined that it has a lowering effect on serum malondialdehyde (MDA), free fatty acid (FFA), triacylglyceride (TAG), total lipid (TL) and low density lipoproteins (LDL) (P<0.05), but it has no effect on other parameters (P>0.05).

Result: As a result, it is thought that the addition of HFE to the drinking water of broiler chickens exposed to HS has no effect on the performance characteristics, HFE has a hypolipidemic effect and would be beneficial in order to strengthen the immune system.

Key words: Broiler chickens, Carcass, Hawthorn fruit extract, Heat stress, Performance, Serum biochemistry.

INTRODUCTION

The increase in temperatures around the world due to global warming and climate change is one of the leading impediments to the poultry sector, as heat stress has a negative impact on the welfare and health of the animals, leading also to economic losses (Bayraktar and Tekce, 2019; Tekce *et al*., 2019). Undesired physiological and biochemical effects are observed in broiler chickens exposed to heat stress (HS), such as a decrease in feed intake (FI), body weight gain (BWG), carcass and suppression of the immune system (Goo *et al*., 2019; Moustafa *et al*., 2021). The modernist approach to the prevention of the unfavorable effects of HS on poultry production tends to focus on diets enriched with phytogenic feed additives (Tekce *et al*., 2020; Bayraktar *et al*., 2021).

Hawthorn (*Crateagus oxyacantha*), a medicinal plant, has strong antioxidant activity due to its presence in different bioactive compounds such as epicatechin, hyperocyte and chlorogenic acid (Wu *et al*., 2020). Hawthorn (*Crateagus oxyacantha*) fruit extract (HFE) has been reported to have antioxidant, antimicrobial and hypolipidemic properties (Aierken *et al*., 2017). Although there are studies examining the effects of hawthorn supplementation on poultry (Zhang *et al*., 2009; Ahmadipour *et al*., 2017; Song *et al*., 2018; Dai *et al*., 2021), no study has been found on the effect on broilers reared under heat stress. The present study evaluates the addition of HFE (*Crataegus oxyacantha*) to the drinking water of broiler chickens subjected to cyclic heat stress on the performance, carcass characteristics and some serum parameters.

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MATERIALS AND METHODS

Ethical approval

This study was carried out pursuant to the approval (dated 18.09.2020 and numbered 2020/13) of the Local Ethics Board for Animal Experiments of the Directorate of Veterinary Control Central Research Institute.

Animals and experimental design

The study was conducted with 300 1-day old Ross 308 male broilers. The study lasted for a total of 42 days, including an initial 7-day acclimatization period and then 35-day growth periods. After the acclimatization period, the chicks were weighed and randomly distributed in 30 wire pens (10 chicks/

pen). The pens measured 121×110×108 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. 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) × 3 (dietary treatments) factorial arrangement from 21 to 42 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 heat stress 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. In heat stress temperature room (HS), 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. In addition, a fluorescent lighting schedule of 24 h light was used during the study with an average light intensity of 40 lux/m^2 (Sarıca *et al*., 2015).

Feed

In the study, broiler starter (0-10 days), grower (11-24 days) and finisher feeds (25-42 days) purchased from a private company were used. All of the broiler chickens consumed the same feeds. The effects of three different HFE levels were examined, with 0, 0.2 and 0.4 ml of extract, purchased form a private company, added to a liter of water. By calculating the amount of extract that broilers/chickens housed in TN conditions would take with the water they would drink in 24 hours, 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 calculated amount of HFE was added to the treatment groups in 100 ml of water and only 100 ml of water was added to the control groups and given to the chickens through one liter drinkers. Following the completion of these, hanging chicken drinkers were put into use and the experiment was continued with fresh water. Consumption of feed and additive-free drinking water was provided *ad libutum* throughout the experiment.

Analytical procedures

Feed analysis

A raw nutrient analysis of the feeds used in the research was conducted using near-infrared spectroscopy device. The ingredients and chemical compositions of basal diet used in the experiment are given in the previous article by Kaya (2023).

Analyzes of hawthorn fruit extract

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 HFE was determined as 1538.94 mg GAE/L. The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) scavenging activity was determined using the method described by Gülçin (2005). The absorbances were measured at 517 nm using a visible spectrophotometer (ethanol was used as blank). In order to determine the scavenging activity of HFE, 3 samples were taken for each method and analyzed and their arithmetic averages were calculated. It was determined that the DPPH scavenging activity of HFE was 51.592 (IC50, µg/ml). Phenolic component profile and organic acid profile analyzes of HFE were determined using the high-performance liquid chromatography (HPLC) method (Coklar and Akbulut, 2017). The analysis of phenolic compounds and organic acid profile was conducted by an Agilent 1260 Infinity Series HPLC system equipped with a G1311C pump, a diode array detector (G1315D, Agilent) and separation was done with a reversed-phase ACE GENERİX5 C18 column (5 µm, 250 × 4.6 mm I.D.). The phenolic compound and organic acid profiles is presented in Table 1.

Determination of performance, carcass characteristics and the weight of visceral

All animals were weighed individually to determine the BWG on days 7, 14, 21, 35 and 42 of the experiment and the amount of feed ingested in each section was recorded for the calculation of the FI and the feed conversion ratio (FCR), which is expressed as the amount of feed consumed per unit of live weight gain. 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. The carcasses of the slaughtered chickens belonging to the experimental groups were kept at +4°C for 24 hours for cold carcass weighing. Carcass characteristics and internal organ weights were measured as a percentage of body weight.

Determination of serum parameters

At the end of the experimental period, blood samples (1 samples/per replicate, 5 chickens/per group, 30 in total) were taken from the vena jugularis. Then, serum samples were obtained by centrifuging the blood samples at 3500 rpm for 10 min. Serum total antioxidant capacity (TAC) was measured using the colorimetric method developed by Erel (2004); serum total oxidant capacity (TOC) was measured using the spectrophotometric method and calibrated with hydrogen peroxide (Erel, 2005); serum malondialdehyde (MDA) were measured using the spectrophotometric method described by Ohkawa *et al.* (1979), serum IgG concentrations were measured using the enzyme-linked immunosorbent assay (ELISA) described by Li *et al*. (2000) and corticosterone

(CORT) were measured using radioimmunoassay kits (Byk-Sangtec Diagnostica, Dietzenbach-Germany; Immulite 2000, DPC, LA) (Sahin *et al*., 2003). Serum levels of calcium (Ca), phosphorus (P) and magnesium (Mg), alanine aminotransaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total lipid (TL), total cholesterol (T-CHOL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose (GLU), total protein (T-PRO), albumin (ALB) and globulin (GLB) parameters were measured with a fully automatic biochemistry device Cobas 8000 (Roche, Germany). Serum-free fatty acid (FFA) and triacylglyceride (TAG) levels were determined using HPLC method (Sherma, 2003).

Statistical analysis

The data derived from the experiments were analyzed with IBM SPSS Statistics (Version 23.0. Armonk, NY: IBM Corp.) following a 2×3 factorial statistical analysis design (General Linear Model procedure). Duncan's multiple range test was used to compare the contributions of different doses of feed additives and an Independent Samples t-test was used to compare the effects of heat stress.

RESULTS AND DISCUSSION

Broiler growth performance

Temperatures outside the thermoneutral range are one of the important environmental factors affecting the performance and health characteristics of broilers (Tekce *et al*., 2020). Growth, feed consumption, live weight gain and feed efficiency of broiler chickens exposed to acute or chronic heat stress may be adversely affected (Goo *et al*., 2019; Moustafa *et al*., 2021). Factors such as the distribution of the high temperatures produced in the gastrointestinal tracts of broilers to peripheral tissues during heat stress, impairment in digestion and absorption and the suppression of appetite-regulating centers in the hypothalamus have been suggested as reasons for decreases in FI and BWG (Marchini *et al*., 2016). In our study, it was determined that the addition of HFE to drinking water had no effect on the growth performance [FI(g), BWG (g) and FCR (g/g)] of broilers raised under heat-stress conditions (Table 2) (P>0.05). While our results are in agreement with some literature data, FCR (Li *et al*., 2009; Sun *et al*., 2013) and FI and BWG (Li *et al*., 2009; Marcinèáková et al., 2011; Sun *et al*., 2013; Song *et al*., 2018) are not in agreement with some literature data (Zhang *et al*., 2009; Ahmadipour *et al*., 2017). The reason for this is thought to be caused by the duration and severity of HS, the extraction method of HFE, the doses added to the diet and the differences in its composition.

Carcass characteristics and the weight of visceral

Heat stress can affect muscle metabolism and visceral organs in animals raised for food production. In our study, it was determined that the addition of HFE to drinking water had no statistical effect on hot and cold dressing and thigh and breast dressing (Table 3) in broiler chickens reared under heat-stress conditions (P>0.05). The addition of different levels of HFE to the drinking water of broiler chickens reared under HS has been found to have no statistical effect on visceral weights (heart, spleen, abdominal fat, liver, bursa fabricus) (P>0.05). The results of our study for carcass characteristics (Marcinčáková *et al.,* 2011; Moustafa *et al*., 2021) showing compatibility, it differs from some research results (Li *et al*., 2009; Zhang *et al.,* 2013; Ahmadipour *et al*., 2017; Hosseini-Vashan *et al.,* 2020). Internal organ weight liver (Dai *et al.,* 2021) bursa fabricus and abdominal fat ratio (Hosseini-Vashan *et al*., 2016) while showing compatibility in terms of their results, they differ from some research results (Zhang *et al.,* 2013; Ahmadipour *et al*., 2017; Hosseini-Vashan *et al*., 2020).

Serum parameters

Digestion and absorption of the consumed nutrients occur depending on the biochemical reactions that may alter according to the animal's breed, health status and

Table 1: Phenolic compound and organic acid profiles of hawthorn fruit extract.

Parameter	Value (mg/L)
Phenolics	
Ascorbic acid	93.63
Gallic acid	254.82
Protocatechuic acid	nd
Catechin	26.13
Hydroxybenzoic acid	nd
Vanillic acid	nd
Gentisic acid	53.85
p- Coumaric acid	31.01
Rutin	102.71
Ferulic acid	nd
Naringin	81.03
o-Coumaric acid	nd
Neohesperidin	91.18
Coumarin	nd
Resveratrol	59.03
Quercetin	81.14
t-Cinnamic acid	52.92
Hesperidin	nd
Alizarin	nd
Flavone	nd
Total	927.45
Organic acids	
Formic acid	6232.13
Ascorbic acid	nd
Lactic acid	nd
Acetic acid	2871.72
Fumaric acid	42.79
Succinic acid	nd
Propionic acid	nd
Total	9146.64

nd not detected.

environmental factors (Bayraktar *et al*., 2023). Stress is an important factor that changes biochemical parameters (Kaya *et al*., 2021). In our study, it was determined that the addition of HFE to drinking water had a lowering effect on the biochemical parameters MDA, FFA, TAG, TL and LDL of broilers raised under heat-stress conditions (P<0.05), but had no effect on other parameters (P>0.05) (Table 4, Table 5). Hyperlipidemia, defined as elevated serum triglyceride and lipoprotein levels, occurs as a result of disorders in the lipid metabolism (Çetin, 2014) and elevated triglyceride and LDL levels and low HDL levels pave the way for hyperlipidemia, leading to a vascular disorder known as atherosclerosis by causing a deposition of lipids beneath the endothelium of vessels. Herbal extracts can regulate serum lipid profili by decreasing lipid oxidation through the phenolic compounds in their content, altering certain biochemical

Table 2: Effect of hawthorn (*Crataegus oxyacantha*) fruit extract supplementation in drinking water on growth performance of broilers reared under heat stress.

		HFE (ml/L water)	FI (g)	BWG (g)	FCR (g/g)
T	TN	0	4416	3066	1.440
		0.2	4316	3078	1.402
		0.4	4306	3049	1.412
	HS	0	4191	2958	1.416
		0.2	4210	2926	1.439
		0.4	4173	2923	1.428
	SEM	29	16	0.007	
T	TN	4346	3064	1.418	
	HS	4191	2935	1.428	
HFE		0	4304	3012	1.420
		0.2	4263	3002	1.421
		0.4	4240	2986	1.428
			p-value		
T			0.01	0.01	0.54
HFE			0.61	0.63	0.90
T*HFE			0.64	0.72	0.30

HFE- Hawthorn fruit extract; T- Temperature; TN- Thermo-neutral; HS- Heat stres; SEM- Standart error of the mean; FI- Feed intake; WG- Weight gain; FCR- Feed conversion ratio (g FI/g WG).

Table 3: Effect of hawthorn (*Crataegus oxyacantha*) fruit extract supplementation in drinking water on carcass characteristics and visceral weights of broilers reared under heat stress (%, Body weight).

		HFE (ml/L water)	HD	CD	TD	BD	Liver	Heart	Spleen	Bursa fabricus	Abdominal fat
T	TN	0	67.8	67.6	26.4	21.4	2.22	0.542	0.096	0.138	1.60
		0.2	68.0	67.2	26.6	22.2	2.30	0.538	0.102	0.148	1.54
		0.4	65.6	65.2	26.0	22.8	2.16	0.530	0.102	0.132	1.56
	HS	0	68.6	67.6	27.8	21.6	2.08	0.584	0.098	0.160	1.40
		0.2	69.6	68.8	27.2	22.2	2.08	0.504	0.086	0.152	1.60
		0.4	67.8	67.2	27.8	20.4	1.98	0.522	0.088	0.152	1.34
	SEM	0.3	0.3	0.2	0.3	0.04	0.117	0.003	0.003	0.05	
T	TN	67.1	66.7	26.3	22.1	2.23	0.537	0.100	0.139	1.57	
	HS	68.7	67.9	27.6	21.4	2.05	0.537	0.091	0.155	1.45	
HFE		0	68.2 [a]	67.6 [a]	27.1	21.5	2.15	0.563	0.097	0.149	1.50
		0.2	68.8 [a]	68.0 [a]	26.9	22.2	2.19	0.521	0.094	0.150	1.57
		0.4	66.7 [b]	66.2 [b]	27.0	21.6	2.07	0.526	0.095	0.142	1.45
						p-value					
T			0.01	0.02	0.01	0.22	0.05	1.00	0.19	0.02	0.27
HFE			0.01	0.02	0.92	0.58	0.54	0.30	0.94	0.51	0.66
T*HFE			0.50	0.24	0.55	0.15	0.93	0.42	0.52	0.42	0.50

HFE- Hawthorn fruit extract; T- Temperature; TN- Thermo-neutral; HS- Heat stres; SEM- Standart error of the mean; HD- Hot dressing; CD- Cold dressing; TD- Thigh dressing; BD- Breast dressing.

[a-b.]Values within a column with different superscripts differ significantly (P<0.05).

and metabolic processes during the dispersion and deposition of lipids in the tissues (Ye *et al*., 2009; Marcinčáková *et al.,* 2011). While the results of the study are compatible with several research results (Marcinčáková *et al.,* 2011; Song *et al*., 2018; Ahmadipour *et al*., 2019; Dai *et al*., 2021), they differ from some of the research results (Ye *et al*., 2009; Xie et al., 2009; Hosseini-Vashan *et al*., 2016). The reason for the difference in the results of the study is thought to be shaped depending on the trial protocol, HFE type and application dose.

Table 4: Effect of hawthorn (*Crataegus oxyacantha*) fruit extract supplementation in drinking water on serum parameters of broilers reared under heat stress.

		HFE (ml/L water)	TAC (µmol/L)	TOC (µmol/L)	MDA (mmol/L)	TIgG (g/L)	CORT (nmol/L)	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	AST (U/L)	ALT (U/L)
T	TN	0	760	2.99	4.99^{c}	1.38	39.2	7.39	4.44	1.97^{bc}	173	6.64
		0.2	765	3.18	4.95^{c}	1.62	44.0	7.46	4.57	2.06^{a}	185	6.80
		0.4	816	3.29	4.82^{c}	1.61	46.0	7.79	4.49	2.04^{a}	153	6.36
	HS	0	678	3.26	7.59^{a}	1.89	54.8	7.40	4.59	2.02^{ab}	176	6.37
		0.2	753	3.09	6.07^{b}	2.18	57.0	7.58	4.67	1.93^{c}	191	6.36
		0.4	797	3.00	5.81^{b}	2.20	57.5	7.87	4.60	2.00^{ab}	187	6.11
	SEM	14	0.06	0.21	0.06	1.5	0.10	0.05	0.01	6	0.07	
T	TN	780	3.15	4.92	1.54	43.1	7.55	4.50	2.02	170	6.60	
	HS	743	3.12	6.49	2.08	56.4	7.62	4.62	1.99	185	6.28	
HFE		0	719^{b}	3.13	6.29^{a}	1.63^{b}	47.0^{b}	7.40	4.51	1.99	175	6.51^{ab}
		0.2	759^{ab}	3.14	5.51^{b}	1.90^{a}	50.5^{ab}	7.52	4.62	1.99	188	6.58^{a}
		0.4	806^{a}	3.14	5.32^{b}	1.91^{a}	51.7^{a}	7.83	4.55	2.02	170	6.24^{b}
							***p*-values**					
T			0.16	0.77	0.01	0.01	0.01	0.72	0.31	0.07	0.23	0.02
HFE			0.04	0.99	0.01	0.01	0.05	0.21	0.71	0.35	0.43	0.05
T*HFE			0.48	0.15	0.02	0.83	0.66	0.97	0.99	0.01	0.47	0.77

HFE- Hawthorn fruit extract; T- Temperature; TN- Thermo-neutral; HS- Heat stres; SEM- Standart error of the mean; TAC- Total antioksidant capacity; TOC- Total oxidant capacity; MDA- Malondialdehyde; IgG- Total immunoglobulin G; CORT- Corticosterone; Ca- Calcium; P- Phosphorus; Mg- Magnesium; AST- Aspartate aminotransferase; ALT- Alanine aminotransferase.

[a-c,] Values within a column with different superscripts differ significantly (P<0.05).

Table 5: Effect of hawthorn (*Crataegus oxyacantha*) fruit extract supplementation in drinking water on serum parameters of broilers reared under heat stress.

		HFE (ml/L water)	ALP (U/L)	FFA (%)	TAG (%)	TL (mg/dl)	T-CHOL (mg/dl)	LD (mg/dl)	LHDL (mg/dl)	GLU (mg/dl)	T-PRO (g/dl)	ALB (g/dl)	GLB (g/dl)
T	TN	0	77.7	18.82 ^{a}	40.35 ^{a}	712 ^{a}	186	71.6 ^{a}	48.9^{d}	156 ^{b}	4.75	1.61	3.14
		0.2	65.4	7.95 ^{b}	36.03 ^{b}	564 ^{b}	187	67.6 ^{b}	55.2^{c}	178 ^{a}	4.90	1.66	3.24
		0.4	51.1	5.27 ^{d}	32.95 ^{c}	464 ^{c}	192	60.3 ^{c}	53.8^{c}	172 ^{a}	5.49	1.66	3.83
	HS	0	61.0	6.42 ^{c}	26.81 ^{d}	364 ^{d}	218	49.9 ^{d}	76.1^{a}	125 ^{d}	4.70	1.59	3.10
		0.2	60.8	6.92 ^{c}	29.41 ^{d}	392 ^{d}	227	49.7 ^{d}	72.7^{b}	129 ^{d}	4.72	1.56	3.16
		0.4	75.1	7.14 ^{bc}	28.36 ^{d}	368 ^{d}	229	48.3 ^{d}	71.3^{b}	141 ^{c}	5.02	1.63	3.38
	SEM	3.5	1.11	1.19	24	5	1.7	2.0	4	0.10	0.02	0.10	
T	TN	64.7	10.68	36.44	580	188	66.5	52.6	168	5.05	1.65	3.40	
	HS	65.6	6.83	28.19	375	225	49.3	73.4	132	4.81	1.60	3.21	
HFE		0	69.3	12.62 ^{a}	33.58 ^{a}	538 ^{a}	202	60.7 ^{a}	62.5	141 ^{b}	4.72 ^{b}	1.60	3.12
		0.2	63.1	7.43 ^{b}	32.72 ^{ab}	479 ^{b}	207	58.7 ^{b}	63.9	154 ^{a}	4.81 ^{ab}	1.61	3.20
		0.4	63.1	6.21 ^{c}	30.65 ^{b}	416 ^{c}	211	54.3 ^{c}	62.5	156 ^{a}	5.25 ^{a}	1.65	3.61
								***p*-values**					
T			0.89	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.23	0.21	0.34
HFE			0.69	0.01	0.04	0.01	0.69	0.01	0.13	0.01	0.05	0.62	0.12
T*HFE			0.06	0.01	0.01	0.01	0.92	0.01	0.01	0.01	0.67	0.57	0.64

HFE- Hawthorn fruit extract; T- Temperature; TN- Thermo-neutral; HS- Heat stres; SEM- Standart error of the mean; ALP- Alkaline phosphatase; FFA- Free fatty acid; TAG- Triacylglycerol; TL- Total lipid; 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).

CONCLUSION

In this study, it was determined that the addition of HFE to drinking water of the broiler chickens exposed to heat stress had not showed a positive effect on the growth performance, carcass characteristics and visceral weights (P>0.05). Serum MDA, FFA, TAG, TL and LDL concentrations were reduced significantly the addition of HFE to stressed groups (P<0.05), but it has no effect on other parameters (P>0, 05). It is thought that the addition of HFE to the drinking water of broilers exposed to heat stress has a hypolipidemic effect and would be beneficial for strengthening the immune system.

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Conflict of interest

The author declares that there is no conflict of interest regarding this study.

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