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The Effect of Supplementation of Betaine on Performance, Carcass Yield and Some Blood Parameters in Broilers*

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Summary: This study aims to investigate the effects of betaine, added to broiler rations at different levels, on fattening performance, carcass, some internal organs weights, and serum parameters. 192 mixed-sex one-day-old broiler chicks were used during the experiment. The experiment was conducted with four groups in total, including one control and three experimental groups. All the groups were divided into four sub-groups, each consisting of 12 chicks, and the total number of chicks in each group was determined to be 48. No additives were added to the feeds of the control group throughout the research. 0.3 g/kg (Bet-0.3), 0.5 g/kg (Bet-0.5) and 0.8 g/kg (Bet-0.8) betaine (Betamar®) were added to the rations of the experimental groups, respectively. Rations were prepared as isocaloric and isonitrogenic. The animals were given feed and water ad-libitum. In the study, The rations containing crude protein (CP) of 23% and 3000 kcal/kg metabolic energy (ME) until the day 1-21 and CP of 20% and 3224 kcal/kg ME until the day 22-42 days were given in the treatment groups. At the end of the study, statistically significant differences were observed in the experimental groups in terms of body weight (BW) and body weight gain (BWG) when compared with the control group. The highest BW and the highest BWG were observed in the Bet-0.8 experimental group. No statistically significant difference was found in terms of feed consumption, feed conversion ratio, carcass characteristics, weights of some internal organs, and serum blood parameters ($P>0.05$). It was concluded that adding 0.8 g/kg betaine to broiler rations increase fattening performance and can be used safely.

Key words: Betaine, blood parameters, broiler, carcass, fattening performance

Broilerlerde Betain İlavesinin Performans, Karkas Verimi ve Bazı Kan Parametreleri Üzerine Etkisi

Özet: Bu araştırma broiler rasyonlarına farklı düzeylerde ilave edilen betainin besi performansı, karkas, bazı iç organ ağırlıkları ve serum parametreleri üzerine etkilerini araştırmak amacı ile yapılmıştır. Deneme boyunca 1 günlük 192 adet karışık cinsiyette broiler civciv kullanılmıştır. Deneme 1 kontrol ve 3 deneme grubu olmak üzere toplam 4 grupta yürütülmüştür. Tüm gruplar 12 civcivden oluşan 4 alt gruba bölünmüş, her grubun toplam civciv sayısı 48 olarak belirlenmiştir. Araştırmada boyunca kontrol grubu yemlerine bir katkı ilave edilmemiştir. Deneme grupları rasyonlarına sırasıyla 0.3 g/kg (Bet-0.3), 0.5 g/kg (Bet-0.5) ve 0.8 g/kg (Bet-0.8) g/kg betain (Betamar®) ilave edilmiştir. Deneme rasyonları izokalorik ve izonitrojenik olarak hazırlanmıştır. Hayvanlara yem ve su ad-libitum verilmiştir. Araştırmada deneme gruplarına 1-21. güne kadar %23 ham protein (HP): 3000 kcal/kg metabolik enerji (ME), 21- 42.güne kadar ise %20HP ve 3224 kcal/kg ME içeren rasyonlar verilmiştir. Araştırma sonunda, kontrol grubuna göre, deneme gruplarında canlı ağırlık (CA) ve canlı ağırlık artışı (CAA) bakımından istatistiksel farklılık görülmüştür ($P<0.05$). En yüksek canlı ağırlık ve canlı ağırlık artışının rasyonlarına 0.8 g/kg betain ilavesi yapılan grupta olduğu belirlenmiştir. Deneme sonu itibari ile yem tüketimi, yemden yararlanma oranı, karkas özellikleri, bazı iç organ ağırlıkları ve serum kan parametreleri bakımından istatistiksel olarak farklılık bulunmamıştır ($P>0.05$). Sonuç olarak; broiler karma yemlerine 0.8 g/kg betain ilavesinin besi performansını olumlu etkileyebildiği ve güvenle kullanılabileceği sonucuna varılmıştır.

Anahtar kelimeler: Besi performansı, betain, broiler, kan parametreleri, karkas

Introduction

After the ban on antibiotics that were used for many years in increasing growth performance in the European Union from January 1, 2006, several feed additives that can be considered an alternative have been used, greatly supporting the developments in the poultry industry (Eklund et al., 2005; Özsoy et al., 2017).

Betaine is a derivative of a commonly found, long-chain glycine amino acid, and it has three methyl groups. It was firstly discovered in sugar beet, and then, found to exist in other plants, animals, and seafood. Many purified forms of betaine (anhydrite monophosphate and hydrochloric betaine) are used today (Eklund et al., 2005). Betaine, a source of methyl, is both taken with feeds and forms as a result of oxidation of choline. As poultry rations are usually formulated to be corn and soybean residues-based, methionine is deficient in poultry animals fed with

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these rations and it becomes necessary to receive it from outside (Eklund et al., 2005). Betaine taken extracellularly or synthesized from choline enables the osmolyte balance of a cell. Also, it enables it to convert into methionine or cysteine by transsulfuration by giving methyl group to homocysteine formed by methionine metabolism (DasSarma et al., 2006; Howe et al., 2008). Another function of betaine is to serve as the donor of methyl groups that are required during the conversion of homocysteine into methionine (Lever and Slow, 2010). Betaine, choline, and methionine are the 3-methyl group providers in the rations of livestock animals. Methionine is primarily used in protein synthesis, while the rest is used for methyl group reactions. After choline is used in the synthesis of acetylcholine, the rest of it can be transformed into the betaine molecule (Niculescu and Zeisel, 2002).

Betaine increased the total body weight and carcass weight by enhancing mineral absorption and retention and enhancing the water retention capacity of the muscle tissue (Esteve-Garcia and Mack, 2000). It was reported that betaine affected carcass quality by reducing the inhibitory effect of pH on enzyme activities (Eklund et al., 2005) and had positive impacts on

performance in poultry if used together with methionine (Esteve-Garcia and Mack, 2000; Schutte et al., 1997). It was also reported that the performance values in broilers increased linearly with adding methionine and betaine (El-Husseiny et al., 2007), on the contrary, McDevitt et al. (2000) reported that the interchangeable use of betaine and methionine in poultry animals would not impact the performance and they could not be used in that way.

This study aimed to evaluate the effects of feed-additive betaine, added to broiler rations at different amounts, on fattening performance, carcass characteristics, internal organ weights and blood parameters and contribute to the literature.

Materials and Method

This study was conducted based on the approval of the Local Ethics Committee for Animal Experiments of Kafkas University dated October 25, 2018, and numbered KAÜ-HADYEK/2018/089. The research was conducted at the poultry unit of Research Farm of the Faculty of Veterinary, Kafkas University. 192 daily Ross-308 broiler chicks were used as the ani-

Table 1. Composition and chemical analysis of experimental diets

Ingredients (%)	Starter Diet (d 1 to 21)	Finisher Diet (d 22 to 42)
Corn grain	50.58	52.75
Soybean meal	30.40	16.75
Wheat grain	6.00	5.00
Bonkalite	5.50	5.50
CDDGS	-	4.10
Corn protein	2.01	4.20
Rice bran	-	4.62
Vegetable oil	1.02	3.00
Di calcium phosphate	1.00	0.60
Meat-Bone meal	2.30	2.30
L-lysine	0.40	0.40
DL-methionine	0.30	0.30
Sodium Bi-carbonate	0.09	0.09
Salt	0.15	0.14
Vitamin-Mineral mix*	0.25	0.25
Multi-Enzyme	0.09	-
Chemical Analyses (%)		
Dry matter	88.15	88.24
Crude protein	23.00	19.99
Crude fiber	2.96	2.72
Crude fat	4.48	4.49
Crude ash	6.13	4.90
Metabolic Energy**(kcal/kg)	3000.10	3224.90
Calcium**	0.98	0.79
Phosphorus**	0.50	0.58

*KAVIMIX VM 214: Vit A: 12000000 IU; Vit D3: 1500000 IU; Vit E: 30000 mg; Vit K3: 5000 mg; Vit B1: 3000 mg; Vit B2: 6000 mg; Vit B12: 30 mg; Folic Acid: 750 mg; Cal. D.Panth: 10000 mg; D Biotin: 75 mg; Cholin Chloride: 375000 mg; Nicotinamid: 40000 mg; Mn: 80.000 mg; F: 40000 mg; Zn: 60000 mg; Cu: 5000 mg; Sn: 100 mg; I: 400 mg; S: 150 mg; Antioxidant: 10000 mg (per 2.5 kg);

**Calculated nutritional values.

CDDGS: Corn dried distiller's grainssolubles.

mal material. The chicks were divided into four main groups including one control and three experimental groups, and each main group was divided into four sub-groups, each of which consisted of 12 chicks. The experiment lasted for 42 days as 7 days of adaptation and 35 days of feeding period. Each group was fed with broiler chick feed (23% CP, 3000 kcal/kg ME) during 0-21st days chick and broiler feed (20% CP, 3224 kcal/kg ME) during 22-42nd days. The nutrients and chemical composition of rations are shown in Table 1. The animals were subjected to group feeding. Feed and drinking water were *ad-libitum*. While no additive was added to the feed of the C group, 0.3 g/kg (Bet-0.3), 0.5 g/kg (Bet-0.5) and 0.8 g/kg (Bet-0.8) betaine were added to the feeds of the experimental groups, respectively. Betaine (Betamar®) used in the study was obtained from the VIMAR A.Ş./İstanbul Corporation. The product contained a minimum 98% Betaine HCL in its chemical composition according to the certified analysis results reported by the producer company (Table 2).

Electric radiant heaters were used to heat the poultry house throughout the study. The temperature of the

and their BWG were calculated. In order to calculate feed consumption (FC), the amounts of feed that animals in each subgroup could consume were put into the feeders and their amounts were recorded. The amount of feed remaining in the feeders in the weekly weights was subtracted from the total feed given to each subgroup and the amount of feed consumed by each subgroup in the previous week was found. This amount was divided by the current number of animals in the subgroups and groups and by the number of days, and FC's were calculated as weekly FC per animal in the groups and subgroups. Feed conversion ratios (FCR) of the groups were calculated by dividing the average amount of feed they consumed between two weighing by the average increase in body weights.

The animals were weighed individually and slaughtered at the end of the study. The chickens were beheaded and the slaughter was performed in this way. After the slaughter, the feathers of the animals were plucked with a plucking machine; the feet and the internal organs were removed. After the slaughter process, carcass weights were determined. Carcass

Table 2. The composition of betaine used in the research

Ingredients	Standard	Result
Appearance	White crystalline powder	
Dry Weight (%)	≤0.5	0.37
Hydrochloric acid (%)	≥%95-98	95.53
Heavy metals (Pb) (%)	≤0.001	≤0.001
Arsenic (As) (%)	≤0.002	≤0.002
Silica %	3	3
pH (25%W/V L/gr, water)	0.8-1.2	1.0

poultry house was kept at 35 °C (± 1) in the first week. Then, it was reduced to 25 °C gradually. The temperature was reduced to 20 °C in the last two weeks of the study and kept at 20 °C until the end of the study. Wood shavings were used as the base material. 24-hours illumination was provided by also utilizing sunlight. The experimental area was divided into equal partitions (1.40 x 1.09 m²) for each subgroup. Wood shavings with a depth of 6 to 8 cm were used as the base material. Chick feeders and drinkers were placed into the partitions during the period of the first 14 days. Then, chick feeders were removed and hanging chicken feeders were placed in the following periods and these feeders and drinkers were used until the end of the experiment. The animals that died throughout the experiment were recorded daily. The amounts of crude nutrients of the rations used in the beginning and end periods of the research were determined according to the methods reported by the AOAC (1990).

The animals were weighed individually at the start of the experiment and in the following weeks. A precision balance (±10 mg) was used in all weighing. The differences between the weights were determined

weights were divided by pre-slaughter weights and carcass yields were calculated. The slaughter process was carried out removing the internal organs of the chickens and weighing the internal organs of each animal using precision balances (± 10 mg). The liver, heart, gizzard, and spleen weights were calculated and recorded.

Statistical analyses

BW, BWG, FC, FCR, performance characteristics, carcass parameters, internal organs, and blood values were analyzed by one-way ANOVA (SPSS 18 portable). Differences between the subgroups were analyzed by Duncan's test.

Results

The weekly body weights and the body weight gain in the study are given in Tables 3. At the end of the experiment, statistically significant differences were found between the BW and average BWG values of the control and the experimental groups in which 0.3 g/kg (Bet-0.3), 0.5 g/kg (Bet-0.5) and 0.8 g/kg (Bet-0.8) betaine was added to the feed (P<0.05).

The mean daily (FC) values of the research groups weekly and the FCR are given in Tables 4. The differences observed in the FC and FCR during the 7-14th

sera of the betaine added mixed feeds are shown in Table 6. The total protein, albumin, globulin, glucose, and uric acid levels in the blood serum were not af-

Table 3. The effect of betaine supplementation on BW and BWG of broiler chicks

BW (g/chick)						
Groups	7 days	14 days	21 days	28 days	35 days	42 days
Control	175.79±0.60	424.50±3.79	828.39±10.28	1311.80±12.95	2068.91±65.27	2591.22±20.44 ^b
Bet-0.3	179.06 ±1.02	436.87±6.09	844.87±5.49	1286.58±23.89	2136.59±73.00	2678.68±53.90 ^{ab}
Bet-0.5	177.18±1.59	425.76±7.46	825.94±13.05	1280.54±24.65	2003.31±2.90	2551.20±53.55 ^b
Bet-0.8	176.66±0.65	430.35±3.68	860.03±19.45	1363.84±32.75	2085.58±32.87	2817.39±88.18 ^a
P	0.079	0.410	0.265	0.121	0.468	0.035
BWG (g/day/chick)						
Groups	7-14. days	14-21. days	21-28. days	28-35. days	35-42. days	0-42. days
Control	35.52±0.60	57.70±1.14	69.05±2.12	108.17±9.03	226.82±5.10	69.01±0.57 ^b
Bet-0.3	36.83±0.75	58.28±0.25	63.1 ±3.91	121.43±8.27	214.44±20.65	71.41±1.56 ^{ab}
Bet-0.5	35.51±0.94	57.16±1.13	64.94±3.44	103.25±3.33	201.70±4.67	67.82±1.55 ^b
Bet-0.8	36.53±0.47	61.43±3.29	71.92±3.06	109.95±8.30	201.04±17.24	75.50±2.25 ^a
P	0.465	0.395	0.253	0.415	0.126	0.036

BW, body weight; BWG, body weight gain.

Results are expressed as mean ± standard error of the mean (SEM).

Differences among groups were not statistically significant (P >0.05)

Groups that were statistically different were indicated by a letter (* P<0.05)

days of the research were eliminated at the end of the experiment and were not found statistically significant.

ected by the betaine addition (P>0.05).

The carcass parameters and liver, heart, spleen, and gizzard weights are given in Table 5. Accordingly, no

Table 4. The effect of betaine supplementation on FC and FCR of broiler chicks

FC (g)						
Groups	7 days	14 days	21 days	28 days	35 days	42 days
Control	76.50±1.32 ^{ab}	123.29±14.77	164.16±4.20	1182.06±9.88	226.82±5.10	154.57±5.13
Bet-0.3	71.57±2.86 ^{bc}	107.05±7.45	168.37±0.18	197.75±5.66	214.44±20.65	151.83±4.55
Bet-0.5	68.56±3.96 ^c	114.12±6.87	163.01±5.17	196.34±3.71	201.70±4.67	148.74±3.78
Bet-0.8	79.61±1.86 ^a	118.38±10.09	161.34±4.23	194.24±12.14	201.04±17.24	150.92±8.34
P	0.02	0.723	0.641	.0566	0.530	0.910
FCR						
Groups	7-14. days	14-21. days	21-28. days	28-35. days	35-42. days	0-42. days
Control	2.15±0.04 ^a	2.12±0.23	2.38±0.12	1.72±0.20	3.22±0.53	2.15±0.04 ^a
Bet-0.3	1.94±0.07 ^b	1.83±0.13	2.70±0.18	1.64±0.09	2.87±0.50	1.94±0.07 ^b
Bet-0.5	1.92±0.06 ^b	1.99±0.08	2.52±0.06	1.90±0.66	2.63±0.24	1.92±0.06 ^b
Bet-0.8	2.18±0.04 ^a	1.93±0.13	2.26±0.15	1.77±0.87	2.08±0.20	2.18±0.04 ^a
P	0.013	0.626	0.196	0.531	0.285	0.013

FC, feed consumption; FCR, feed conversion ratio.

Results are expressed as mean ± standard error of the mean (SEM).

Groups that were statistically different were indicated by a letter (* P<0.05)

significant difference was found between the experimental groups in terms of slaughter, carcass weight, hot carcass yields, and internal organ weights (P>0.05).

Statistical evaluations on the total protein, albumin, globulin, glucose, and uric acid levels in the blood

Table 5. The effect of betaine supplementation on carcass parameters of broiler chicks

Groups	CarcassParameters						
	SW (g)	WC (g)	DP (%)	Liver (g)	Heart (g)	Spleen (g)	Gizzard (g)
Control	3016.00±65.24	2285.18±39.70	75.82±0.55	49.50±2.32	14.36±0.97	2.22±0.97	38.40±2.95
Bet-0.3	3058.37±26.95	2356.18±24.24	77.04±0.40	52.20±1.89	13.95±0.57	1.95±0.57	36.61±2.21
Bet-0.5	2899.62±59.25	2235.12±40.17	77.12±0.61	57.09±2.99	14.43±0.94	1.88±0.17	37.08±1.64
Bet-0.8	3009.21±28.29	2337.06±43.35	76.41±0.25	55.22±2.89	15.82±0.43	1.88±0.18	38.60±1.57
P	-	-	-	-	-	-	-
	0.149	0.123	0.257	0.191	0.474	0.550	0.895

SW: Slaughter weight; WC: Warm carcass; DP: Dressing percentage; Results are expressed as mean ± standard error of the mean (SEM).

Differences among the groups were not statistically significant ($P > 0.05$).

Table 6. The effect of betaine supplementation on blood parameters of broiler chicks

	Blood Parameters (mg/dl)				
	Total Protein	Albumin	Globulin	Glucose	Uric Acid
Control	3.00±0.04	0.96±0.22	2.06±0.31	220.10±8.96	6.78±0.56
Bet-0.3	2.94±0.09	0.95±0.04	1.97±0.63	228.20±3.97	6.09±0.47
Bet-0.5	3.01±0.10	1.03±0.20	2.11±0.33	227.70±6.19	6.65±0.32
Bet-0.8	2.92±0.12	1.02±0.03	2.00±0.52	222.00±14.24	7.82±0.37
P	0.874	0.116	0.158	0.898	0.065

Results are expressed as mean ± standard error of the mean (SEM).

Differences among the groups were not statistically significant ($P > 0.05$).

Discussion and Conclusion

At the end of the study, statistically significant differences were found between the growth performances of the control and experimental groups. The highest increase in body weight was observed in the Bet-0.8 experimental group, in which 0.8 g/kg betaine was added to the rations. The results of this study are consistent with those of the study on the effects of adding only betaine or together with methionine to the broiler rations on performance parameters (Hassan et al., 2005; Honarbakhsh et al., 2007; Zhan et al., 2006). Hoşgör (2005) reported that a 500-mg/kg betaine addition to the drinking water of broilers had no effect on BW on the 12th day; however, it provided an increase in BW on the 47th day. Besides, the findings on the increase in body weights obtained in the study are similar to the findings of several other studies (Attia et al., 2005; El-Husseiny et al., 2007; Virtanen 1995). Sakomura et al. (2013) reported that the betaine addition improves the characteristic and structural functions of the intestinal epithelial cells and in this way, nutrients can be more easily digested. Chand et al. (2017) reported that betaine addition to the rations of broilers significantly affected body weight gain, the highest body weight was observed in the group in which 2 g/kg betaine was added to the rations, and the more the dose of betaine the more the body weights increase compared with the control group. The researchers stated that the increase in

body weight was because of the fact that betaine increased the digestibility of specific nutrients as stated by Eklund et al. (2006).

On the other hand, this research is not consistent with the studies in which betaine was used together with ionophore anticoccidials (Matthews et al., 1997; Waldenstedt et al., 1999) and the studies reporting that betaine addition did not have any effect on BW and BWG (Esteve-Garcia and Mack, 2000; Pirompu et al., 2005; Uzunoğlu and Yalçın, 2019).

Although betaine increases energy availability, it needs energy for its excretion as it contains nitrogen. Fernández-Fígares et al. (2002) stated that there was a positive linear correlation between the levels and impacts of betaine. Xu et al. (1999) emphasized that the addition of betaine over 0.08% to the rations reduced its effectiveness. The addition of pure betaine to the betaine-rich mixed feeds had a positive effect on growth performance (Cromwell et al., 1999). The reason for the difference in the effects of betaine was attributed to many factors (Esteve-Garcia and Mack, 2000; Matthews et al., 1997). Regarding the results of the present study and some other studies, the difference between them is thought to originate from the composition of betaine, its usage, its applications at different doses, and using different breeds of animals in the studies.

In this study in which betaine was added to the rations at different amounts, a statistically significant difference was observed between the FC and FCR values in only between 7-14th days. However, this difference disappeared during the following weeks of the experiment and no statistically significant difference was found. Our study is consistent with the results of the reported researchers (El-Husseiny et al., 2007; Esteve-Garcia and Mack, 2000; Jahanian and Rahmani, 2008; Pirompu et al., 2005; Uzunoğlu and Yalçın, 2019) and El-Husseiny et al. (2007) results showing that betaine addition (0.5, 0.75, and 1 mg/kg) to the rations that contained methionine at different rates did not affect feed consumption in broilers. However, these experimental results differ from those of the studies reporting increased feed consumption, when compared with the control group (Awad et al., 2014; Sakomura et al., 2013). Similarly, they do not comply with the reports stating that betaine increases FC and FCR (Attia et al., 2005; Chand et al., 2017; Hassan et al., 2005; Honarbakhsh et al., 2007).

In consequence of the findings obtained at the end of the experiment, no statistically significant difference was found between the groups in terms of carcass parameters, and heart, liver, spleen, and gizzard weights in the control and experimental groups. The present study is similar to those by (El-Shinnawy 2015; Honarbakhsh et al., 2007; Uzunoğlu and Yalçın, 2019) reporting that betaine addition did not affect carcass yield and heart, liver, spleen, and gizzard weights.

However, it was reported that betaine addition to broiler rations at a lower level (1 g/kg) did not affect the carcass yield when compared with the control group, yield ratio increased significantly with the increase in the dose (1.5 and 2.0 g/kg), this increase was caused by the osmotic effect of betaine, which increased dropsy (Chand et al., 2017). In especially some studies, betaine was reported to have a positive impact on the breast meat yield (Attia et al., 2005; Esteve-Garcia and Mack, 2000; Noll et al., 2002; Remus 2001). In the same way, betaine addition was reported to increase carcass yield and muscle tissue protein ratio in poultry (El-Shinnawy 2015; Hassan et al., 2005; Jahanian and Rahmani, 2008; Wang et al., 2004). Betaine takes effect by reducing carcass fat content and enhancing carcass lean meat percentage. The positive effects of betaine addition on carcass yield are thought to depend on the methyl donor function in protein metabolism.

In the experiment, serum, total protein, albumin, globulin, glucose, and uric acid levels of the groups were not affected by the betaine addition. The results of the experiment are similar to those of the studies reporting that betaine addition to rations does not affect blood serum parameters in broilers (Attia et al.,

2005; Baghaei et al., 2011). In some studies conducted with betaine addition, it was reported that serum triglyceride level was lower than the other groups (Uzunoğlu and Yalçın, 2019), betaine significantly reduced plasma triglyceride levels, did not affect plasma cholesterol and LDL levels, and increased HDL levels (Jahanian and Rahmani, 2008). This decrease in triglycerides was reported to be because of the use of betaine in the synthesis of carnitine and lecithin molecules (Saunderson and Mackinlay, 1990). Also, Hassan et al. (2005) contradict the statement that betaine addition at different levels in broilers increased serum total protein and albumin levels when compared with the control groups. The researchers reported that this result proved that betaine is a methyl donor in protein metabolism. Zhan et al. (2006) reported that betaine significantly reduced serum uric acid concentration in broilers. El-Husseiny et al. (2007) stated that the increase in doses of betaine addition to the mixed feeds of broilers increased serum total protein and globulin levels, and plasma components except for total protein were not affected in the second trial.

It was concluded that betaine addition to the mixed feeds took positive effects on performance and could be used at an amount of 0.8 g/kg.

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