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Effect of GM maize on metabolism and performance of chicken

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Genetically modified (GM) maize and conventional maize were tested experimentally to determine their effect on chicken serum biochemical parameters and metabolism. A total of 600 one day old parent stock chickens of breed White Plymouth Rock were divided into 2 experimental groups: K - diet based on traditional maize (control); D - a diet based on GM maize (experimental). During the experimental period (17 weeks), population vibility and feed consumption were monitored daily. At the age of 4, 8 and 17 weeks all birds were individually weighed. Blood samples for biochemical analyses were collected from chickens at 4 weeks and 12 weeks of age (n = 6). Analytical studies did not show significant changes in biochemical serum parameters in group D chickens. A significant difference between the groups of chickens receiving GM and traditional maize was found only by the content of lipids after 4 weeks (P < 0.05) and cholesterol after 12 weeks of the experiment (P < 0.05). Also noted increasing level of circulating immune complexes in serum of chickens that consumed feed from GM maize relative control at 32.2% (P < 0.05) after 4 weeks of the experiment. However, the studied indicators of metabolism and immune response in birds were within the physiological range. Certain fluctuations in the parameters during the experiment were observed in both groups, which indicates that there is no connection between this fact and the feeding factor. In addition, optimal results were obtained in terms of feed conversion and body weight of poultry, regardless of the feeding ration. The body weight of chickens at the age of 17 weeks in both groups was in the range of 1,862.4–1,895.6 g, feed consumption per 1 kg of body weight gain was 3.65–3.76 kg, the livestock viability – 96.6-97.3%. The results of the study do not indicate any danger to the metabolism and health of the bird due to the use of genetically modified feed, as no statistically significant differences within the studied parameters were observed. The noted certain small deviations fell within the limits of normal variations of the considered indicators and, thus, had no biological or toxicological value.

Keywords: metabolism, growth performance, feeding, GM maize, chicken

1 Introduction

Genetically modified crops have become widespread among growers because they have advantages over isogenic counterparts in terms of plant protection from insect damage, weed control safety, and other benefits for maximum yields on minimum crops. In Ukraine there is no law that regulates the sphere of circulation of GMOs, although the prospects for the development of the agricultural sector with the use of GM crops are obvious. It should be noted that unofficial estimates Ukraine has large areas of GM soybean, up to 25% of GM material is detected in maize hybrids. Monitoring of one hundred samples maize from the fields of Dnipropetrovsk, Kiev and Cherkasy regions showed 25 cases of GM hybrids Bt176, MON 810 and GA21 (Volosyanko at al., 2019). Despite the fact that the safety of GM crops is globally approved, there are still some problems.

Genetically modified (GM) maize and soybeans are extensively studied by scientists from different countries for their equivalence to traditional crops in terms of chemical composition and fodder value. Numerous studies have shown that their inclusion in the feed of both broiler chickens and hens did not have a negative impact on the safety of livestock, live weight gain, egg production, feed conversion (McNaughton et al., 2011; Řehout at al., 2009; Tan at al., 2012), the number and diversity of microorganisms in the intestine (Lu at al., 2015).

Data on the effect of transgenic feed components on productivity, biochemical metabolic parameters,

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antioxidant and immune systems of animals and poultry are quite limited and contradictory. The most data resources indicate the safety and equivalence of genetically modified crops for animal compared to conventional varieties from which they were obtained. So, independent animal testing have recently confirmed the absence of toxicity of MON 810 GM maize in subchronic and chronic toxicity studies (Petrick at al., 2019). During long-term feeding of GM maize to pigs, their live weight, feed conversion and viability were minimally different from the control indicators (Chen et al., 2016). Scholtz et al. (2010) found no deterioration of the immune system in quail when fed on genetically modified maize. The experiment Szymczyk et al. (2018) Indicators metabolism in rats fed a diet with conventional or genetically modified (MON 810) maize for 16 weeks was similar. The authors also no found significant differences in the body weight and the relative weight of the organs of rats, some histopathological changes in the liver, kidney and spleen were not related to nutrition.

Genetic modification allows the introduction of new genes, information and products. However, these new genes, which give the plant the expected and improved properties, can also cause unwanted metabolic changes and become a source of stress in living organisms. So, Yalçin et al. (2018) observed elevated levels of malondialdehyde liver and kidneys of rabbits treated with GM maize for three generations, which is an indicator of oxidative damage. At the same time, feeding Transgenic Hybrid Corn (VIP3A) broiler chickens for 50 days did not have a significant effect on a number of metabolic function indicators compared to non-GM corn (Brake et al., 2005). Consequently, transgenic maize did not differ from non-GM maize in terms of short-term feeding of chickens, as there was no apparent disturbance in metabolic activity.

Thus, the ambiguity of the results necessitates further comprehensive research on the effect of genetically modified feed on metabolic parameters and the immune system of animals. In addition, most studies on the effects of GM feed on the health and blood parameters of poultry have been performed in broiler chickens, which hold no more than 42 days. Therefore, it is interesting to study the effect of feeding genetically modified maize on the young of the parent stock, the growing period of which is at least 17 weeks.

Based on this, the aim of this article was to study the effect of feeding genetically modified maize in comparison with isogenic maize on metabolic processes and growth parameters of chickens of the parent stock of the dualpurpose breed White Plymouth Rock. We hope that results obtained will provide new data on the biological effects of transgenic maize on poultry and contribute to a more comprehensive study of the possible adverse effects of GM crops on animal health.

2 Materials and methods

2.1 Ethical considerations

Experimental studies involving poultry were approved and endorsed by the Commission on Bioethics of the State Poultry Research Station NAAS (Ukraine) and conducted in accordance with the standards of breeding, keeping and feeding, as well as the recommendations of the European Convention for the protection of vertebrate animals used for experiments or other scientific purposes (Strasbourg, 1986).

2.2 Birds, housing and experimental diets

The experiment was performed on chickens of the factory line G2 of dual purpose breed White Plymouth Rock in the experimental base of the State Poultry Research Station NAAS (Ukraine). 600 one day old sexed chickens (females) were randomly divided proportionally into two experimental groups depending on the diet, one of which (control – K) received feed without genetically modified components, the other (experimental – D) – with genetically modified maize. Chickens were kept in separate sections on the floor on a deep litter at a planting density of 5–5.5 animal units m⁻² in compliance with the recommended technological parameters. The duration of the experiment was 17 weeks.

MON 810 GM maize was used for the experiment, obtained by inserting the gene of the soil bacterium Cry1A (b), which is resistant to European corn borer. The control was the traditional (unchanged) variety of maize DMS 2510. These two varieties of maize were grown in the same ecological conditions. The grain collected from MON 810 and DMS 2510 was strictly separated, avoiding any possibility of movement or contamination. Feed from MON 810 and DMS 2510 was also prepared and stored separately. Each diet was evaluated by polymerase chain reaction (PCR) to confirm the absence (control) or presence (in the experiment) of the Cry1A gene (b). The nutritional value of transgenic and standard maize did not differ significantly. Both diets were isoprotein and isoenergetic, similar in all major nutrient parameters and biologically active substances (Table 1).

2.3 Performance parameter

Chickens were weighed individually at 4, 8, and 17 weeks of age. Livestock viability and feed consumption were monitored daily, as well as live weight gain and feed conversion.

Components (%)	Age of chickens/group					
	1–8 weeks		9–17 weeks			
	К	D	К	D		
Maize	45.29	45.29	40.00	40.00		
Wheat	12.00	12.00	22.87	22.87		
Sunflower meal	16.00	16.00	5.00	5.00		
Soybean meal	15.00	15.00	9.30	9.30		
Fishmeal	5.00	5.00	1.00	1.00		
Alfalfa flour	2.90	2.90	4.00	4.00		
Wheat bran	-	-	13.00	13.00		
Monocalcium phosphate	1.10	1.10	1.30	1.30		
Chalk	1.40	1.40	2.10	2.10		
Premix	1.00	1.00	1.00	1.00		
Salt	0.10	0.10	0.30	0.30		
Lysine	0.15	0.15	0.08	0.08		
Methionine	0.06	0.06	0.05	0.05		
Calculated nutrient composition						
Crude protein	20.00	20.00	14.60	14.60		
Crude fat	5.30	5.30	5.00	5.00		
Crude fiber	4.80	4.80	6.50	6.50		
Calcium	1.12	1.12	1.20	1.20		
Phosphorus	0.76	0.76	0.70	0.70		
Lysine	1.10	1.10	0.70	0.70		
Methionine + cystine	0.75	0.75	0.50	0.50		
Metabolic energy (kcal kg-1)	2,902.00	2,902.00	2,680.00	2,680.00		

 Table 1
 Ingredients, nutrient composition and metabolic energy of the experimental diet (%)

K = control group; D = experimental group

2.4 Sample collection and biochemical analysis

For biochemical analyzes during the experiment, 4 and 12 weeks after the start of feeding the experimental feed, blood was taken from 6 chickens from each group. Approximately 3 ml of blood samples were taken without the anticoagulant heparin from the axillary vein using a sterile syringe in simple tubes. All samples were collected at the same time (9:00 to 10:00) to minimize any changes in blood chemicals caused by circadian rhythms. Blood collection tubes were stored on ice in cool containers to avoid protein denaturation and delivered to the laboratory within 2 hours after blood collection. The serum was separated after centrifugation at 3,000 rpm for 15 minutes.

Selected biochemical parameters of blood serum (cholesterol, uric acid, lipids, malonic dialdehyde (MDA), the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were measured on the basis of photometric detection using commercial test kits (SPE "Filisit diagnosis", Ukraine). The concentration of biochemical components was calculated according to the manufacturing instructions. The optical density of the compounds obtained in color reactions was measured using a photoelectric colorimeter "FEK-56".

The activities of AST and ALT in blood serum were determined transamination reaction with transaminase serum and formation of pyruvate, the number is expected to color reaction with 2.4 dynitrophenylhidrazine. The content of lipids in the serum was determined by reaction with a sulfophosphovaniline reagent. Cholesterol levels were measured by color reaction with acetic anhydride in the presence of acetic and sulfuric acids. The content of MDA was determined by color reaction with thiobarbituric acid. The concentration of uric acid in the serum was determined in a color reaction with a phosphorus-tungsten reagent.

Lysozyme activity of blood serum (LABS) was determined using a spectrophotometer "SF-26" by the change in optical density of the medium as a result of the ability of blood lysozyme to lyse the test culture of *Micrococcus lisodecticus* in 0.5% sodium chloride solution. The concentration of circulating immune complexes (CIC) was determined by a method based on selective precipitation of antigen-antibody complexes with 3.5% solution of polyethylene glycol M-6000 (PEG) with subsequent photometric determination of precipitate density.

All analytical studies were conducted in the feed quality assurance laboratory of the State Poultry Research Station NAAS (Ukraine).

2.5 Statistical analysis

Growth test data, blood biochemical parameters were analyzed using one-way analysis of variance (ANOVA procedure) according to the following model:

$$y_i = \mu + a_i + e_i$$

where:

 y_i – growth parameters, biochemical parameters of the blood of chickens with the *i*-th diet (1 – GM maize, 2 – isogenic maize); μ – average value; a_i – effect of the *i*-th diet (1 – GM maize, 2 – isogenic maize); e_i – random error. Results were analyzed by post hoc Tukey test. The significance level for all tests was P <0.05

3 Results and discussion

Feeding chickens with genetically modified maize during the experiment did not have a clear effect on growth, development and metabolism of poultry. Indicators of growth and development of poultry are given in Table 2. Body weight of chickens of both groups did not deviate from the normative indicators for young dual purpose poultry and was at the level of 499.2–514.1 g at the age of 4 weeks, 1,169.3–1,193.4 g – at 8 weeks and 1,862.4– 1,895.6 g – at the end of cultivation. At the same time, the body weights of chickens fed GM maize compared to the control group (isogenic variability) were higher, 3% and 2.1% at weeks 4 and 8, respectively, but 1.7% lower at 17 weeks. These deviations were not reliable (P >0.05) and were within individual fluctuations.

Since the chickens of both groups received the same feed rate, respectively, its costs per 1 kg of growth in the first 4 and 8 weeks were lower in the experimental group by 3.2-3.7%, and for the entire period of the experiment (0-17 weeks) - by 2.9%. The following results on the feeding of chickens with GM feed were confirmed in studies on the feeding of quails for 10 generations of feed containing GM maize (Sartowska et al., 2015). Another experiment, which lasted for four generations, also did not show a significant effect of feeding Bt-maize on feed consumption of chickens, young and laying hens, live weight of poultry compared to the isogenic analogue (Halle & Flachowsky, 2014). There was also no effect of the presence of genetically modified maize and soybeans in broiler feeds on their growth, nutrient uptake, carcass weight and meat quality (Zhang et al., 2019). In addition, the authors could not find convincing evidence of adverse health effects directly related to the consumption of genetically modified foods. In our experiment in all studied periods, the safety of young animals was high and almost did not differ between groups. In the first 4 weeks of rearing there was a slight advantage of the experimental group on this indicator (by 0.6%), for 8 weeks of life the preservation of the bird was the same in both groups – 99%. As a result, the overall safety of chickens for the whole period was higher by 0.7% in the group that consumed feed with genetically modified maize – 97.3% against 96.6% in the control.

The absence of negative effect of feeding GM maize during 17 weeks of growing on the indicators of preservation, growth and feed conversion in the experimental birds is confirmed by similar results of biochemical studies in both groups. Assessing the biochemical parameters of blood serum which characterize the metabolism in birds (Table 3), we found that a statistically significant difference between groups of chickens was only on the cholesterol content after 12 weeks of the experiment (P <0.05) and lipid concentration after 4 weeks (P <0.05).

Table 2Dynamics of body weight, the viability of chicks and feed consumption

ltem	n Age of chickens/group					
	4 weeks		8 weeks		17 weeks	
	К	D	К	D	К	D
BW	499.2 ±8.23	514.1 ±7.37	1,169.3 ±15.68	1,193.4 ±11.64	1,895.6 ±20.91	1,862.4 ±16.16
FCR	2.16	2.09	2.45	2.36	3.76	3.65
LV	99.0	99.6	99.0	99.0	96.6	97.3

K – control group; D – experimental group; BW – body weight (g); FCR – feed conversion ratio (g diet g weight gain⁻¹; PV – livestock viability (%)

Item	Age of chickens/group				
	4 weeks		12 weeks		
	К	D	К	D	
Uric acid (mmol I-1)	0.319 ±0.025	0.351 ±0.005	0.455 ±0.030	0.475 ±0.030	
Cholesterol (mmol I-1)	1.92 ±0.018	2.19 ±0.012	1.12 ±0.005 ^b	1.39 ±0.001ª	
Lipids (%)	2.32 ±0.31 ^b	3.15 ±0.20ª	3.35 ±0.47	2.60 ±0.44	
AST (nMol (s • l)-1)	254.0 ±14.00	267.0 ±5.00	360.0 ±12.00	318.0 ±26.00	
ALT (nMol (s • l)⁻¹)	248.0 ±57.00	161.0 ±28.00	202.0 ±19.00	196.0 ±32.00	
MDA (µMol I ⁻¹)	2.31 ±0.486	3.19 ±0.484	2.17 ±0.317	2.38 ±0.257	

Table 3Biochemical parameters of chicken blood serum (M \pm m; n = 6)

K – control group; D – experimental group; AST – aspartate aminotransferase activity; ALT – alanine aminotransferase activity; MDA – malonic dialdehyde; a, b – values in the same row (within the age range) with different superscripts differ significantly (P < 0.05)

In the assessment of lipid metabolism of the greatest clinical importance is the determination of serum cholesterol. In our experiment the cholesterol level in chickens fed GM-based feed was 2.19 mmol l-1 at 4 weeks and 1.39 mmol l⁻¹ at 8 weeks. At the same time, we observed an increase in serum cholesterol levels of chickens fed transgenic maize feed relative to control. At 4 weeks, it was significantly higher than the control indicator by 14%, at 12 weeks of the experiment there was a probable increase in serum cholesterol levels of experimental chickens by 24% (P < 0.05) relative to control. Nevertheless, in our studies, the cholesterol level in poultry fed GM maize feed did not deviate from the physiological limit which, according to various sources, was 1–5.4 mmol l⁻¹ (Ritchie et al., 1999; Stevens, 1996). This corresponds to the data obtained in the experiment on broiler chickens fed MON 810 maize (Czerwiński et al., 2015).

It is known that under any stress (loads) there is an accumulation of lipids in the blood. The lipid content in the blood of experimental chickens after 4 weeks of consumption of feed with genetically modified maize reliable exceeded the control value by 35.8% (P < 0.05). Subsequently, it was observed a decrease of the lipid level in the experimental group and an increase in the chickens of the control group, which consumed food with conventional maize. Therefore, at 12 weeks advantage for this parameter was in chickens of the control group (22.4%). Since fluctuations in the parameters during the experiment were observed in both groups, it can be assumed that there is no connection between this fact and the feeding factor. Our data also do not contradict the results of studies of the effect of maize Bt176 on physiological characteristics in poultry, which did not reveal significant differences in metabolism and productivity between birds that consumed common unmodified maize and GM maize (Aeschbacher et al., 2005).

The main end product of purine and protein metabolism in birds is uric acid, in particular, it is synthesized from ammonia, because the enzymes of the ornithine cycle are absent. According to our data, the level of uric acid in the serum of chickens fed based on GM maize, during the experiment did not deviate significantly from control, the differences between groups (from 4.4 to 10% depending on the age of chickens) were incredible. Some increase with age of this indicator in both groups (by 35.3–42.6%) could show a violation of protein metabolism in poultry, but in both cases the content of uric acid in the serum did not exceed the norm that poultry is 0.15-0.48 mmol I-1 (Ritchie et al., 1999). In our opinion, such minor changes in this substance because of the experiment indicate the absence of a significant effect of consumption of GM feed on the processes of protein metabolism in chickens. Similar results were obtained in an experiment on studying the effect of transgenic maize on the physiological characteristics of laying hens (Gao et al., 2014).

The condition of the liver can be judged by the amount in serum ALT, AST, alkaline phosphatase, bilirubin and others. The degree of endogenous intoxication in chickens after feeding GM maize was evaluated by the activity of aminotransferases in the serum, because the increase in their activity is one of the first biochemical tests to diagnose endotoxin and indicates destructive processes in the liver. According to our data, there was a slight increase in the activity of AST in the serum of chickens of the experimental group compared to the control by 5.1% and a decrease in ALT activity by 35.1% after 4 weeks from the start of the experiment. During the experiment, the activity of AST in both groups increased by 16–29.4% and there was an increase in ALT activity in the experiment by 17.9%, but the obtained deviations are not significant. That is, no significant differences were found in the activity of controlled liver enzymes ALT, AST in the serum of chickens fed GM maize and chickens fed

Item	Age of chickens/group			
	4 weeks		12 weeks	
	К	D	К	D
CIC, optical units	0.31 ±0.041 ^b	0.41 ±0.025°	2.46 ±0.43	3.10 ±0.68
LABS (%)	74.05 ±2.89	75.36 ±4.22	70.32 ±0.59	70.63 ±1.73

Table 4Indicators of immune reactivity in chickens (M ±m; n = 6)

K – control group; D – experimental group; CIC – circulating immune complexes; LABS – lysozyme activity of blood serum; a, b – values in the same row (within the age range) with different superscripts differ significantly (P <0.05)

traditional maize, and our results confirm the results of many previous experiments. The experiment on laying hens examined the activity of transaminases (ALT and AST) and found that none of the enzymes showed significant differences between the control group that consumed feed with normal maize and the group fed transgenic maize (Gao et al., 2014). Feeding for 10 generations of feed with a high content of genetically modified maize Bt 176 did not have a significant impact on the health of quails, including indicators of aminotransferase activity (Korwin-Kossakowska et al., 2016).

The content of MDA in the blood is a relatively nonspecific indicator of lipid peroxidation (intensity of lipid peroxidation). On the other hand, the analysis of this indicator is still one of the best predictions of oxidative stress, as it is usually very well related to other markers. Taking into account that the system of antioxidant defense of the organism first reacts to the negative influence of various factors, during the experimental period the content of MDA in the serum of chickens of the studied groups was controlled. In our studies, after 4 weeks of the experiment, the spontaneous accumulation of MDA in the serum of chickens when fed GM-based feed compared to the control increased by 38%, at 12 weeks there was a significant increase in this control (by 74%) and the difference decreased to 9.7%. Higher levels of MDA in the serum of experimental birds may indicate the activation of lipid peroxidation or a decrease in antioxidant protection of the body because of the negative effects of feeding GM food. However, these deviations are unlikely, were within the physiological norm that poultry is 1.5–3.5 µmol l⁻¹ (Lumeij, 1997) and did not contradict similar indicators obtained in studies on broiler chickens (Řehout et al., 2009), laying hens (Gao et al., 2014) and quails (Flachowsky et al., 2005).

Table 4 shows the indicators of immune reactivity in chickens of the experimental groups. Integral index of natural resistance of the organism are bactericidal activity, which is caused by the presence of blood compounds that neutralize microbial cells. High bactericidal activity of blood serum is associated with the content of lysozyme, which has cytolytic properties

against microorganisms. It is able to mobilize other nonspecific factors of protection of an organism and is one of important indicators of a humoral link of immunity. The study of LABS levels in chickens in our experiment showed high rates in both groups, which were maintained in the range of 70.32-75.36%. During the experiment, there was a decrease in this indicator in the control by 3.73%, in poultry of the experimental group, fed feed with the inclusion of GM maize, by 4.73%. At the same time, the LABS in chickens that consumed feed with transgenic maize, both at 4 weeks and 12 weeks of age was almost at the level of analogues in the control. The results of our studies do not contradict the indicators of the immune response under the influence of genetically modified feed, studied in quails (Scholtz et al., 2010) and broiler chickens (El-Kelawy et al., 2018).

Among the factors of nonspecific protection of animals, CIC occupy one of the key positions – they are able to affect the function of lymphocytes, macrophages and, thus, to participate in the regulation of the immune response. In our studies, the serum CIC level of chickens in the experimental group that ate feed with transgenic maize was higher in all study periods than in the control group of chickens that ate feed with normal maize. After 4 weeks of the experiment, an increase in this indicator relative to control by 32.2% (p < 0.01) was recorded, and by the 12th week of the experiment, this advantage decreased to 26%. There was an increase in the level of CIC during the experiment in both groups in 7.6-7.9 times, which corresponds to the age of the bird and most likely not associated with the use of genetically modified components. Based on the fact that the level of formation of immune complexes is an indicator of the degree of pathological reactions in the body, we determined an increase in CIC and some weakening of lysozyme activity in the serum of chickens of both groups in this case can be considered a significant induction of cellular and humoral immunity. Similar conclusions were made when studying the effect of genetically modified soybeans and maize on the body of broiler chickens (Czerwiński et al., 2015). Our data are also confirmed by the results of a study over 4 generations of the effect of feeding transgenic maize on laying hens (Halle & Flachowsky, 2014). In general, most studies have not shown the immunosuppressive effect of genetically modified components in feed on broiler chickens and laying hens, although different levels of their introduction have been used.

Thus, according to the results of the experiment on chickens of the parent stock of the dual purpose breed White Plymouth Rock for 4 months no negative effect of genetically modified maize MON 810 on growth and development, metabolism, immunity was revealed. No dietary adverse effects on bird health have been reported. The detected deviations of some indicators indicate the need to study the long-term effects of genetically modified feed on the body of poultry for an objective conclusion. These results indicate the equivalence of GM maize and isogenic control maize for poultry feeding. This is in line with previous literature reviews on the impact of feeding GM crops on their health and productivity (Snell et al., 2012; Swiatkiewicz et al., 2014; de Vos & Swanenburg, 2018).

4 Conclusion

According to the results of experiment, it can be concluded that the introduction of genetically modified maize MON 810 into the feed for chickens at the level of 40–45% does not affect the body's metabolism and immune functions, and also does not impair the intensity of growth and development of birds. Young animals of the parent stock of the dual-purpose breed White Plymouth Rock fed GM maize for 17 weeks from the one day old showed the similar viability, growth and feed conversion as chickens that ate conventional maize feed. Biochemical studies did not reveal significant fluctuations in metabolism in the body of experimental birds under the influence of GM components of feed. Certain responses of the bird during the experiment, such as some increase in MDA, total lipids and cholesterol in the serum of chickens, may not be of biological importance, as they do not seem to affect the overall health of the bird and its development. That is, transgenic maize is equivalent to conventional isogenic maize for feeding to repair young of dual purpose chickens.

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