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The Influence of Hydrated Aluminosilicate on Biochemical and Haematological Blood Parameters, Growth Performance and Carcass Traits of Pigs

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Abstract: The aim of the study was to investigate the influence of dietary inclusion of the hydrated aluminosilicate ATN at the level of 5 g kg^{-1} diet on haematological and biochemical parameters of blood and productive performance of growing pigs. The major contents of ATN are clinoptilolite and montmorillonite. Fifteen pigs fed basal diet (Control group) and fifteen pigs fed diet with 5 g kg^{-1} added ATN (ATN group) were used in this experiment. The following indices in whole blood were determinated; hematocrit, haemoglobin level, total leucocyte count and activity of antioxidative enzymes in hemolysate. In the blood serum the activity of selected enzymes, metabolic parameters, electrolytes and thyroid hormone status was estimated. Growth rate, feed conversion ratio, carcass weight, dressing percentage, chemical composition of muscle, lean meat and fat content of primal cuts were also estimated. ATN decreased concentration of urea nitrogen and total cholesterol and increased the concentration of sodium and calcium and ALT activity in blood serum. Daily weight gain and feed conversion ratio were positively affected by addition of ATN. The inclusion of ATN did not influence slaughter parameters. Pigs fed a diet with ATN had increased water content and water holding capacity and decreased fat content in muscle.

Key words: Clinoptilolite, phyllosilicate, pig growth, blood serum, cholesterol, Serbia

INTRODUCTION

Most of clays and zeolites are hydrated and composed mainly of aluminum and silica and belong to the group of aluminosilicates. Phyllosilicate clays are crystalline, hydrated aluminosilicates containing alkali and alkaline earth cations and have layered structure. The phyllosilicates could vary in their composition from one phyllosilicate to another, depending mainly on the interchangeable ions that may be contained within its structure. Montmorillonite, main constituent of phyllosilicate ore bentonite is trimorphic phyllosilicate formed by a 2:1 condensation of layers with octahedrally coordinated aluminum sandwiched between two layers of tetrahedrally coordinated silica, possesses exchangeable sodium or calcium cations and has expandable sheets (Phillips *et al*., 1993; Serwicka and Bahranowski, 2004). Zeolites are crystalline, hydrated aluminosilicates of alkali or alkaline earth cations having an infinite, open, three-dimensional honeycomb structure and are able to gain and lose water reversibly and exchange extra framework cations without changing its crystal structure. Clinoptilolite is the most abounded member of this group (Mumpton, 1999; Papaioannou *et al*., 2005). Hydrated aluminosilicates both clays and zeolites have been used in a broad array of applications in animal health and nutrition. The addition of 5-50 g kg^{-1} hydrated aluminosilicates to the diet of food animals has been reported to improve growth and feed utilization and to reduce the incidence and severity of diarrhea in animals (Vrzgula and Bartko, 1984; Mumpton, 1999; Trckova *et al*., 2009). Some zeolites improve egg shell quality and reduce dyschondroplasia in chickens (Trckova *et al*., 2004; Shariatmadari, 2008) but decrease dietary phosphorous utilization (Thilsing-Hansen *et al*., 2002; Eraslan *et al*., 2005). Dietary additions of aluminosilicates have been shown to alter tissue mineral concentrations and have potential value in protecting animals from tissue accumulation of ions of heavy metals (Pond and Yen, 1983; Jain, 1999). The physiological effects of the natural zeolites appear to be related to their high cation-exchange capacity that affects tissue uptake and utilization of ammonium ions as well (Ly *et al*., 2007). Hydrated aluminosilicates have been shown to reduce the absorption of radionucleotides from feed by animals (Chelishchev, 1995; Ahman, 1996). Several studies (Harvey *et al*., 1993; Kececi *et al*., 1998; Pimpukdee *et al*., 2004; Bailey *et al*., 2006) have

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demonstrated that hydrated aluminosilicates commonly used as anticaking agents for animal feeds significantly diminish the adverse effects of aflatoxins in animals. Aluminosilicates are also effective as slow release carriers for many drugs (Dyer *et al*., 2000; Kelly *et al*., 2004).

The objective of the present study was to evaluate effects of Antitoxic Nutrient (ATN) based on natural occurring hydrated aluminosilicates on haematological and serum biochemical status and growth and carcass performance of pigs.

MATERIALS AND METHODS

Animal feeding and housing: Fifteen crossbred (Landrace x Yorkshire) pigs of both sexes from sows fed basal diet (Control group) and 15 pigs from sows fed diet with 5 g kg^{-1} added ATN (ATN group) with an equalized starting live weight of about 14 kg were used. Each group contained eight gilts and seven castrated males. Pigs from control group were fed the basal diets and ATN group were fed basal diets with 5 g kg^{-1} added ATN throughout the whole experiment. ATN is a fine powder containing mostly zeolitic ore (With >90% of clinoptilolite) and bentonite (With >83% of montmorillonite), together with small amounts of activated charcoal (Ratio 60:20:1/ zeolite:bentonite:charcoal). The basal diets mainly based on corn, barley and soybean meal were formulated according to the nutrient requirements for pigs (Table 1). The feed was tested for possible residuals of lead, cadmium, mercury, mycotoxins, antibiotics, coccidiostats and pathogen microorganisms and was found to be free of the above substances. Pigs were offered feed *ad libitum* according to a three-phase programme with transition from starter to grower when the average BW was 35 kg and from grower to finisher when the mean BW was 70 kg. Feed composition and analysis are shown in Table 1. All pigs had free access to water from a nipple adjacent to the feeder. The animals were housed in individual pens in a commercial grower house on a fully slatted concrete floor and an automatically controlled natural ventilation and heating system were in operation. Individual pigs weights were recorded every 14 days and feed intake was recorded daily to calculate Average Daily Gain (ADG) and Feed Conversion Ratio (FCR).

Parameter measured: Blood samples for haematological and biochemical analyses were taken from anterior vena cava at day 120th from animals. Heparin was used as an anticoagulant and noncoagulated blood was tested for Hemoglobin (Hb) concentration, Total Leucocyte Count (TLC) and Hematocrit (Ht). Blood Hb concentration was determined by the cyanomethemoglobin procedure (Wintrobe, 1965). Micro Wintrobe hematoctit tubes and a hematocrit centrifuge were used to determine the Ht. Protein content of blood serum was determined by the method of Bradford (1976) with bovine serum albumin as a protein standard. Concentrations of glucose, urea nitrogen, creatinine, total cholesterol, total triglyceride and activities of Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline Phosphatase (ALP), Amylase (AMY) and Gamma Glutamyl Transferase (GGT) in the blood serum were measured by use of a clinical chemistry analyzer (Microlab 200, Merck). Inorganic phosphorus, calcium, magnesium, iron and chlorides were determined with commercially available kits (Randox laboratories, Ltd, UK). Concentrations of sodium and potassium were measured by flame atomic absorption spectrophotometry (Spectra AA-10, Varian, Australia). Triiodothyronine (T_3) and Thyroxin (T_4) estimations followed the procedure for Radioimmunoassay (RIA) kits supplied by the INEP-diagnostics, Serbia.

Table 1: Ingredients and chemical composition of basal diets

Live weight range	12-35 kg	35-60 kg	60-100 kg
Corn (g kg^{-1})	625.0	590.0	560.0
Barley (g kg^{-1})	150.0	200.0	250.0
Soyabean meal (g kg^{-1})	200.0	180.0	160.0
Sodium chloride (g kg^{-1})	5.0	5.0	5.0
Dicalcium phosphate (g kg^{-1})	5.0	10.0	10.0
Calcium carbonate (g kg^{-1})	5.0	5.0	5.0
Premix[a] (g kg^{-1})	10.0	10.0	10.0
Crude protein (CP) (g kg^{-1} DM)	191.1	162.4	143.7
Ether extract (g kg^{-1} DM)	74.1	67.2	653.0
Crude fibre (g kg^{-1} DM)	48.2	59.0	65.2
Ash (g kg^{-1} DM)	68.5	68.3	74.0
Calcium (g kg^{-1} DM)	7.7	7.1	5.5
Total phosphorus (g kg^{-1} DM)	6.4	5.8	5.9
Sodium (g kg^{-1} DM)	2.1	2.2	1.7
Lysine (g kg^{-1} DM)	11.9	9.2	6.8
Methionine and cysteine (g kg^{-1} DM)	7.6	5.1	5.3
Metabolizable energy (MJ kg^{-1})	13.8	13.5	13.2

[a]Commercial vitamin and trace mineral premixes with adequate amounts of active substances for every phase of growing. DM = Dry Matter

Superoxide Dismutase (SOD) activity was determined in the blood hemolysate according to McCord and Fridovich (1968). The Catalase (CAT) activity in hemolysate was assayed by the method of Clairborne (1986). The utilization of hydrogen peroxide by CAT in the samples was measured spectrophotometrically (Jenway 6505 UV/Vis., UK) as decrease in optical density at 240 nm. The activity was expressed as U g^{-1} Hb. The Glutathione Peroxidase (GSH-Px) in hemolysate was determined by the method of Paglia and Valentine (1967). The rate of oxidized glutathione formation was measured spectrophotometrically at 340 nm with NADPH. The enzyme was expressed as U g^{-1} Hb. Glutathione-disulfide Reductase (GR) activity in hemolysate was determined as per method described by Glatzle *et al*. (1974). The

reduction of oxidized glutathione in the presence of NADPH was measured spectrophotometrically at 340 nm. The activity was expressed in U g^{-1} Hb. The Glutathione S-Tansferase (GST) activity in serum was evaluated using 1-Chloro-2,4 Dinitrobenzene (CDNB) as substrate as previously described by Habig *et al.* (1974). The formation of adduct of GSH-CDNB (2,4-dinitro phenyl glutathion) was monitored by estimation of the increase in absorbance at 340 nm against a blank with a spectrophotometer. The activity was expressed as U mg^{-1} protein.

At slaughter age when attaining an average body weight of 110 kg, all pigs were transported to a slaughterhouse within a farm property. The pigs were killed by exsanguinations after electrical stunning. Scalding, dehairing and evisceration were performed according to conventional procedure. The eviscerated carcass was split longitudinally through the vertebrae midline prior to entry into the chiller and hot carcass (Including feet and head) weight was recorded. After chilling for 24 h at 2°C the carcass was weighed and the both sides of each carcass were divided into primal cuts and shoulder, loin, neck and ham were further analyzed. All dressed cuts were weighed and dissected into lean muscle tissue plus intramuscular fat, subcutaneous fat, bone and skin. Only the fat and lean muscle results are presented in this study. Muscle samples for chemical analysis were collected from the Musclus longissimus dorsi from the last rib and the pH, content of fat ether extract, protein, ash and water holding capacity of the meat was run using international procedures of AOAC (2002).

Statistical analysis: All results were expressed as mean± Standard Error (SE). The t-test was used for the evaluation of differences between control and ATN-supplemented animals (Statistica 6.0 in 2001). Statistical comparisons were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

Effects of dietary treatments on serum chemistry parameters, concentrations of serum macro- and microelements and levels of plasma T_3 and T_4 are shown in Table 2. Serum chemistry values of pigs fed ATN were similar to those of control pigs. Compared to controls, serum concentrations of urea nitrogen and cholesterol were decreased ($p<0.05$) in pigs fed ATN. Serum sodium and calcium levels in pigs fed ATN were significantly higher ($p<0.05$) then in those of control group. Concentration of potassium, magnesium, phosphorus, chlorides and iron did not differ among treatments. Plasma T_3 and T_4 were not significantly affected ($p>0.05$) by dietary treatments.

Table 2: Effects of dietary supplementation of ATN on blood serum biochemical and physiological parameters of pigs

Experimental group	Control	ATN
Glucose (mmol L^{-1})	4.94±0.1100	5.11±0.1400
Total protein (g L^{-1})	68.65±2.1500	72.16±1.5900
Urea N (mmol L^{-1})	5.24±0.6500[a]	3.66±0.1500[b]
Creatinine (µmol L^{-1})	133.07±5.7200	129.48±3.1400
Total holesterol (mmol L^{-1})	2.57±0.1500[a]	1.86±0.1500[b]
Triglyceride (mmol L^{-1})	0.41±0.0300	0.40±0.0200
Sodium (mmol L^{-1})	141.77±2.6500[a]	151.58±1.4700[b]
Potassium (mmol L^{-1})	6.54±0.1800	6.77±0.1300
Magnesium (mmol L^{-1})	1.52±0.0800	1.60±0.0600
Chloride (mmol L^{-1})	103.58±0.8200	103.82±0.4500
Calcium (mmol L^{-1})	2.49±0.0400[a]	2.63±0.0400[b]
Inorganic phosphorus (mmol L^{-1})	3.31±0.0600	3.47±0.0800
Iron (µmol L^{-1})	27.77±2.6400	29.39±1.7600
T_3 (nmol L^{-1})	1163.22±10.510	12308.17±11.290
T_4 (nmol L^{-1})	49433.68±480.24	50844.95±673.84

The data are mean values±SE of 15 animals in each group (n = 15). [a,b]Values without the same superscripts differ significantly ($p<0.05$); T_3 = Triiodothyronine; T_4 = Thyroxin

Table 3: Effects of dietary supplementation of ATN on serum enzyme activites and haematological parameters of pigs

Enzymes	Control	ATN
AST (U L^{-1})	150.00±6.6100	163.16±6.6300
ALT (U L^{-1})	55.17±2.7800[a]	67.39±2.7400[b]
ALP (U L^{-1})	294.22±16.940	323.28±18.770
AMY (U L^{-1})	1702.82±137.65	1809.21±170.02
GGT (U L^{-1})	34.28±2.6200	38.67±2.0300
SOD (U mg^{-1} Hb)	10.47±0.3500	10.89±0.2000
CAT (U mg^{-1} Hb)	37.84±1.9500	38.82±1.1700
GR (U mg^{-1} Hb)	10.70±0.8000	10.03±0.7000
GST (U g^{-1} protein)	37.07±3.6700	40.77±8.0500
GSH-Px (U mg^{-1} Hb)	6.71±0.2200[a]	7.75±0.3000[b]
Hb (g L^{-1})	117.02±2.2100	121.06±2.1700
Ht (%)	35.52±1.0800	37.33±0.6000
TLC (×10^9 L^{-1})	24.10±2.3400	30.11±2.2900

The data are mean values±SE of 15 animals in each group (n = 15). [a,b]Values without the same superscripts differ significantly ($p<0.05$); ALT = Alanine amino transferase; AST = Aspartate amino Transferase; ALP = Alkaline Phosphatase; AMY = Amylase; GGT = Gamma Glutamile Transferase; SOD = Superoxide Dismuase; CAT = Catalase; GR = Glutathione-disulfide Reductase; GST = Glutathione S-Transferase; GSH-Px = Glutathione Peroxidase; Hb = Hemoglobin; Ht = Hematocrit and TLC = Total Leucocyte Count

Data shown in Table 3 show the effects of dietary treatments on serum enzyme activities and haematological parameters of blood. Feeding ATN resulted in a significant ($p<0.05$) increase in ALT activity. Activity of ALP was also increased in serum of pigs fed ATN although not significantly ($p>0.05$). There were no differences in the activities of serum AST, AMY and GGT. Serum GSH-Px activity was significantly ($p<0.05$) higher in pigs fed ATN supplemented diet in comparison to control group. Activities of other measured serum antioxidative enzymes (SOD, CAT, GST and GR) were similar in both

Table 4: Effects of dietary supplementation of ATN on growth and carcass performance of pigs

Factors	Control	ATN
Daily weight gain (g)	0.720±0.055[a]	0.785±0.042[b]
Feed conversion ratio (kg kg^{-1})	2.470±0.180[a]	2.260±0.120[b]
Carcass (kg)	87.340±2.480	89.320±1.180
Dressing (%)	76.760±2.070	79.140±0.700

The data are mean values±SE of 15 animals in each group (n = 15). [a,b]Values without the same superscripts differ significantly (p<0.05)

Table 5: Effects of dietary supplementation of ATN on tissue composition of pigs

	Relative content in primal cut		Relative content in chilled carcass	
Compsition (%)	Control	ATN	Control	ATN
Ham	-	-	25.20±0.32	25.32±0.39
Lean	61.94±0.96	63.70±0.90	15.62±0.42	16.09±0.38
Fat	17.66±1.14	16.57±0.86	4.44±0.26	4.18±0.23
Sholder	-	-	15.35±0.34	15.07±0.19
Lean	55.03±0.79	57.80±1.93	8.49±0.18	8.71±0.32
Fat	17.83±1.90	15.83±0.61	2.76±0.33	2.38±0.09
Loin	-	-	14.65±0.47	15.16±0.24
Lean	29.56±1.34	30.37±1.38	4.31±0.15	4.60±0.17
File	5.02±0.31	5.17±0.23	0.73±0.03	0.80±0.04
Fat	26.72±1.65	26.17±0.63	3.94±0.34	3.96±0.11
Neck	-	-	7.98±0.35	8.04±0.09
Lean	49.62±3.26	47.67±1.00	3.82±0.14	3.84±0.10
Fat	12.12±2.43	13.59±1.68	0.97±0.20	1.09±0.13

The data are mean values±SE of 15 animals in each group (n = 15)

Table 6: Effects of dietary supplementation of ATN on chemical composition of pigs muscle longissimus dorsi

Experimental group	Control	ATN
Water (%)	71.69±3.32[a]	74.39±2.65[b]
Fat (%)	6.72[a]±0.11	4.01±0.14[b]
Protein (%)	20.45±0.17	20.41±0.25
Ash (%)	1.04±0.02	1.11±0.03
pH	5.88±0.07	5.92±0.06
Water holding capacity (%)	18.66±1.22[a]	29.36±2.14[b]

The data are mean values±SE of 15 animals in each group (n = 15). [a,b]Values without the same superscripts differ significantly (p<0.05)

experimental groups. The values of analyzed haematological indices determined in blood of pigs of both experimental treatments were within reference range. The feeding of 5 g kg^{-1} ATN additive did not appear to influence either health or appetite of the pigs. Significant improvement (p<0.05) was noticed in ADG and FCR fed supplemented ATN (Table 4). Pigs offered the diets supplemented with ATN grew significantly faster and had a significantly lower FCR than their control counterparts. Carcass and dressing percentage were not affected by dietary treatment.

Relative content of primal cuts (Shoulder, loin, neck and ham) in chilled carcass and relative content of lean meat and fat in cuts were not affected by dietary treatment (Table 5). All measured parameters were equalized among groups.

The effects of ATN supplementation on pH and chemical composition of the Musclus longissimus dorsi are shown in Table 6. Normally, muscle pH is about 7 and rapidly declines after slaughter. In this trial the meat pH, 24 h after slaughter, reached an average of 5.90. Treatment had no significant effect on this variable. The chemical characteristics of the muscle showed significantly (p<0.05) higher values for water content in animals fed diet supplemented with ATN. Supplemental ATN also significantly (p<0.05) increased water holding capacity and decreased level of fat in pigs muscle and did not affect level of protein and ash in meat.

The addition of dietary ATN to the basal diets resulted in decreasing serum concentration of total cholesterol. These results are in agreement with research of Alexopoulos *et al.* (2007) and Prvulovic *et al.* (2007) on pigs. At the same time, these results are in disagreement with the research of Harvey *et al.* (1993) on chickens. In their research concentration of serum total cholesterol increased (Although not significantly) by dietary supplementation of zeolitic ores. The mechanism of this effect is not clear yet. Magnesium silicate sepiolite could promote increase of the digesta retention time in the gut of pigs and poultry. Longer retention times in the gastrointestinal tract and increases in the microbial activity in small intestine could promote deconjugation of bile acids (Ouhida *et al.*, 2000). One possible explanation is that clinoptilolite, a main constituent of ATN, adsorbs bile acid salts or cholesterol on its surface in the digestive tract, decreasing the level of total cholesterol in the blood serum thereby influencing the need for their synthesis *de novo*. Thus, lowering of serum cholesterol could be the result of its increased utilization for bile acid salts synthesis. Poulsen and Oksbjerg (1995) found that the addition of clinoptilolite to the diet changed the pattern of nitrogen excretion towards increased nitrogen excretion with faeces and decreased nitrogen excretion in urine. This implies that in present experiment binding of ammonium ions to ATN in digestive tract reduces their absorption and thus also the conversion of ammonium to urea which resulted in decreased urea concentration in blood serum. Alexopoulos *et al.* (2007) also recorded decreased concentration of serum urea when pigs were fed diet supplemented with clinoptilolite. Clinoptilolite, a major constituent of ATN has high selectivity for ammonium cations and relatively low selectivity for calcium and sodium cations (Mumpton, 1999) which could account for the significantly higher serum concentrations of sodium and calcium. ATN absorbed ammonium cations in the gastrointestinal tract and released calcium and sodium exchangeable cations from its own structure thus inducing a shift in serum electrolyte status. It is important to note, however that concentrations of all measured electrolytes were within the reference range for pigs and that mentioned shift in electrolyte status probably did not induce disturbance of physiological homeostasis of

the animals. Concentration, type and purity of used aluminosilicate and animal species could interfere with these effects.

Supplemental ATN at level of 5 g kg^{-1} diet increased serum ALT and ALP activitiy. This is partially in agree-ment with the results of Ward *et al.* (1991) and Kubena *et al.* (1990) who reported that supplementation with zeolite A and hydrated sodium calcium aluminosilicate improved the activity of ALP in the blood serum. This effect could be connected with better calcium utilization in the digestive tract. Higher serum activity of ALT could be an indication of tissue (Especially liver) damage if it was accompanied with increased activity of other serum enzymes, especially AST. Activities of all other measured enzymes were within the reference range for pigs and similar to the control group. However, detected ALT activity in serum of pigs of ATN group was within reference range for pigs and does not indicate pathological changes in organism.

Reactive Oxygen Species (ROS) such as superoxide O_2^-, hydroxyl radical OH^- and hydrogen peroxide H_2O_2 are constantly generated in cells through some metabolic processes and inflammation. The increase in intracellular levels of ROS to such a level that cellular antioxidant defenses are insufficient to maintain these harmful molecules below a toxic threshold level is generally referred to as oxidative stress. Oxidative stress and oxidative damage are considered to play a role in the early stages of some pathophysiological processes. Oxidative stress could be prevented by the action of the enzymatic and chemical antioxidative defenses. The enzymes that provide first line of defense against ROS include SOD, CAT and GSH-Px. GR and GST has also significant role in antioxidative defense (Cnubben *et al*., 2001; Nordberg and Arner, 2001). The results show that values for antioxidative enzyme activity (Especially for the GST, key enzyme in antioxidative protection) in blood were not affected by dietary treatment and that animals were not exposed to xenobiotics nor prooxidants. Recorded increased activity of selenium-dependent GSH-Px could be connected with better selenium utilization promoted by addition of ATN. The haematological indices (Hb, Ht and TLC) were not affected by supplementation of ATN which is in agreement with results of Alexopoulos *et al.* (2007) on pigs and Kececi *et al.* (1998) on chickens.

Different experiments with animals fed hydrated aluminosilicates in their diets have given variable results for animal performance. Positive effects have been reported in pigs by Vrzgula and Bartko (1984) and Alexopoulos *et al.* (2007) and in poultry by Harvey *et al.* (1993). In contrast, Poulsen and Oksbjerg (1995) and Fokas *et al.* (2004) found little or no effects of aluminosilicates on animal performance. Type and concentration of hydrated aluminosilicates supplemented to the diet could be the most important factors affecting hydrated aluminosilicates performance in animals. Nutritional level, environmental condition and animal species may also interfere with these effects. The present study found that oral ATN administration of 5 g kg^{-1} diet had positive effect on pigs growth performance and feed conversion ratio.

At present there is very limited information about meat quality and carcass characteristics of animals fed hydrated aluminosilicates. ATN seems to positively and significantly affect chemical composition of muscle, lowering fat content and increasing water holding capacity of meat. The reason for these effects are still not clear. Protein and ash content of muscles were not affected by dietary treatment. Carcass characteristics and composition were similar in both experimental groups.

CONCLUSION

Results obtained in the present experiment confirm a positive effect of hydrated aluminosilictaes on growth parameters of pigs and that dietary addition of 5 g kg^{-1} ATN has no adverse effects on biochemical and physiological homeostasis of animals. The decrease of fat content and improved water holding capacity in muscle is also of great interest. Further researches are proposed to clarify the casual mechanisms likely involved in the use of aluminosilicates to improve the nutritive value of different diets.

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