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Diet supplementation with *Saccharomyces cerevisiae* influences the electrophoretic parameters in blood in young Charolaise bulls

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Abstract

Introduction: The objective of the research was to investigate the effect of *Saccharomyces cerevisiae* supplementation on some acute-phase proteins, haptoglobin and all electrophoretic parameters in young Charolaise bulls. **Material and Methods:** Sixty bulls were divided into two equal groups: the control group (CG) receiving the base diet without yeast supplementation and the diet supplementation group (YG) receiving the base diet with 5g of *Saccharomyces cerevisiae* supplementation. The base diet was total mixed ration allocated at 11.85 kg per animal per day. Blood samples were collected from all bulls on day 0 before the start of the diet supplementation, and on days 20 and 40 after the start. Total proteins, albumin, globulin fraction (α_1-, α_2-, β_1-, β_2- and γ-globulins), albumin : globulin ratio (A : G) and haptoglobin were determined. **Results:** Two-way analysis of variance showed a significant effect of the yeast feeding time on all studied parameters except α_2-globulins in both groups. The YG showed a higher average concentration of total proteins, albumin and A : G and a lower average concentration of γ-globulins and haptoglobin than the CG. **Conclusion:** These results indicated the beneficial effect of the *Saccharomyces cerevisiae* on the inflammatory status of the young bulls, which showed an adequate response in serum levels of the acute-phase proteins tested.

Keywords: total proteins, serum protein fractions, haptoglobin, steers, *Saccharomyces cerevisiae*.

Introduction

Livestock systems aim to improve animal production and welfare. Nowadays, zootechnical systems aim to enhance animal growth and productivity to maximise profit in the shortest time practical. In such systems, beef steers are fed a minimum of roughage and a high amount of concentrate. A high amount of highly fermentable substrates in the diet can lead to imminent rumen dysfunction, induced by an alteration of the microbial population with ruminal inflammation and metabolism disorders (22). Recent studies have demonstrated the direct and indirect positive effects on performance and overall benefits to animal health of the yeast *Saccharomyces cerevisiae*, which have led to an increase in its use as a supplement in the feeding of beef and dairy cattle (8, 23), other ruminants (27), poultry (2) and monogastric animals (7).

Ruminants obtain energy from plant compounds through microbial fermentation in the rumen. This process can be potentiated by probiotics. Among probiotics, live yeast cultures have received particular attention from the research community. Yeasts are an important source of products with probiotic activity in livestock systems and provide livestock producers with a replacement for sub-therapeutic antibiotic supplementation, thereby mitigating the potential negative effects of morbidity in order to guarantee the health status of animals as well as maximise their profitability.

Saccharomyces cerevisiae feed supplementation is known as a tool to improve some aspects of both animal health and the yield per animal to the farm. A large amount of highly fermentable substrates in the diet can lead to imminent rumen dysfunction, induced by an alteration of the microbial population which causes

ruminal inflammation and metabolism disorders (22). The wide use of yeast supplementation proceeds from yeast's bioregulatory actions, which occur through various mechanisms, including microbial antagonism, stimulation of the immune system, attachment and removal of pathogens, boosts to the activity of bacteria-specific enzymes, inhibition of toxins, and modulation of cytokines and oxygen removal, which are useful to minimise the proliferation of aerobic bacteria (22). The most prominent yeast used as a feed additive in livestock is *Saccharomyces cerevisiae*, which is rich in digestible proteins, vitamins (vitamin B6, biotin, thiamine, riboflavin, nicotinic acid and pantothenic acid), magnesium and zinc (15).

The ruminal ecosystem benefits from *Saccharomyces cerevisiae* diet supplementation, which promotes homeostatic balance, improves the immune system and/or produces antimicrobial substances in both ruminants and non-ruminants interacting with immune cells, and exploits the antioxidant properties of the yeast (2).

In the digestive system of animals which receive yeast supplementation, the addition increases microbial activity by means of the action of its vitamin and enzyme constituents. In particular, several effects induced by yeast supplementation in steers were shown through the evaluation of rumen function, hepatic markers, growth performance, feed intake and digestibility, immunity stimulation and reproductive performance (3, 7). It has been reported that *Saccharomyces cerevisiae* supplementation has a positive effect on ruminal fermentation, increasing the level of volatile fatty acids and ruminal ammonia concentration and consequently modifying the gut ecology, that likewise it has a desirable effect on food digestibility, increasing body weight, and that it also modulates the inflammatory response (16). Moreover, the function of probiotics has been studied for their protection against enteric disease, reduction of mortality, lessening of shedding of *Escherichia coli*, and growth promotion. However, several studies reported no effects of *Saccharomyces cerevisiae* supplementation on the growth rate or performance of steers (6). Productivity gains, feed intake improvements and the well-being of animals can be undermined by several stressors caused by mismanagement at all stages of cattle development (20). In particular, various management errors, notably overcrowding, low fodder intake and exposure to pathogens, can compromise the immune system by triggering inflammatory responses (11). These harmful conditions have a negative impact on the immune system and wellness, and animals become susceptible to several pathogens. Yeast supplementation may be prescribed to mitigate these negative effects; however, responses to yeast supplementation are highly inconsistent as suggested by available works in the literature (6, 24). They are inconsistent probably because several factors bear on cattle welfare regarding genetics, feeding history, diet and metabolic adaptation, gastrointestinal microbiota, stress, infections, the yeast dose administered and the productivity stage of the animals (6, 24).

Physiological or pathological conditions in cattle are shown by different serum protein concentrations. Their evaluation by electrophoresis is a valuable and commonly used laboratory diagnostic tool in veterinary medicine (4). Albumin is the most osmotically active serum protein and is a carrier of many substances. Globulins are a heterogeneous group of serum proteins that includes antibodies and other inflammatory molecules, haemostatic and fibrinolytic proteins, and carriers of lipids, vitamins and hormones. These examples show that serum proteins have multiple functions. The change between physiological and pathological conditions can cause shifts in albumin and globulin concentrations, and their monitoring is an indispensable husbandry tool.

Considering that the acute-phase response is an expression of bulls' wellness and reflects their inflammation status, and that minimising provocation of the response is crucial for their performance, the aim of this study was to evaluate the effect of diet supplementation with *Saccharomyces cerevisiae* on the serum levels of protein fractions and haptoglobin in young Charolaise bulls using electrophoresis.

Material and Methods

Animal and experimental design. Sixty clinically healthy, 10-month-old Charolaise bulls with a body weight between 501 and 535 kg were enrolled in this study. The enrolled animals were randomly selected from a farm located in the north-east of Italy. All animals were subjected to a clinical examination before the start of the study and at every time point throughout all experimental periods. The animals were divided into two equal groups (n = 30) and housed in different pens. Thirty bulls were the diet supplementation group (YG), which received daily *Saccharomyces cerevisiae* diet supplementation for 40 d, and the other thirty were the control group (CG), which received only the base diet. Food and water were available *ad libitum*. Animals were fed a total mixed ration once a day (9.00 a.m.). The *Saccharomyces cerevisiae* given as supplementation was the NCYC Sc 47 E1702 strain, of which viable cells were produced by batch fermentation in a growth medium typical of those used for the industrial production of yeasts. The concentration of *Saccharomyces cerevisiae* was 1,010 colony-forming units/g.

The feed ingredients and analytical composition of the diet used are shown in Table 1. The total mixed rations were analysed using near-infrared reflectance spectroscopy (NIRSystem 5000; FOSS Italia, Padova, Italy). A 5g mass corresponding to 5 dL of liquid commercial *Saccharomyces cerevisiae* feed supplement was premixed with concentrate before preparation of the 11.85 kg/head provision of the total mixed rations. The paddock was equipped with headlocks that reserve a specific amount of room for an animal and prevent

dominant bulls pushing down the rail and displacing other bulls. After each feeding time, all mangers were checked to verify that all food and supplementation had been consumed. The yeast supplementation was palatable and did not influence food assumption.

Table 1. Feed ingredients and chemical analysis of total mixed rations provided to the experimental animals

Feed ingredients (kg per day per head)	
BULL 100 11.11*	0.50
Corn gluten feed	0.60
Alfalfa hay	1.00
Corn	2.00
Dry pulp	1.00
Straw	0.80
Corn silage	5.65
Soybean meal	0.30
Total	11.85
Dry matter (%)	57.17
Chemical composition	
Crude protein (%)	13.16
Ethereal extract (%)	3.11
Fibre (%)	14.98
Ash (%)	6.07
Neutral detergent fibre (%)	38.93
Starch (%)	32.52
Ca (g)	69.47
P (g)	24.94

* – Bull 100 11.11 protein, vitamins and minerals premix: vitamin A (169,000 UI/kg), vitamin D3 (16,900 UI/kg), vitamin E (416 mg/kg), vitamin B1 (42 mg/kg), vitamin B12 (0.22 mg/kg), choline (845 mg/kg), niacinamide (1,793 mg/kg), manganous sulphate (191 mg/kg), manganous oxide (381 mg/kg), zinc chelate of amino acids (5,954 mg/kg), zinc oxide (742 mg/kg), copper sulphate pentahydrate (216 mg/kg), cobalt carbonate (2.2 mg/kg), potassium iodide (14.7 mg/kg), urea (49,500 mg/kg)

All treatments were carried out and housing and animal care conditions provided in accordance with the standards recommended by Directive 2010/63/EU for animal experiments (14).

Sampling and laboratory analysis. Blood samples were collected by tail venepuncture using 22 G × 25 mm needles into 10 mL serum vacuum tubes with clot activator (Vacutainer; BD Diagnostics - Preanalytical Solutions, Plymouth, UK). Sampling was carried out in both groups on day 0 (t0) immediately before the supplementation of the experimental diet, and 20 (t1) and 40 (t2) days after its start. The samples were allowed to clot for 2 h at 4°C before centrifugation at 1,350 × *g* for 10 min.

The concentration of total proteins, albumin, globulin fractions and haptoglobin was assessed in the obtained serum samples. Serum total protein concentration was assessed with a commercial kit by means of an automated ultraviolet spectrophotometer (Slim; SEAC, Florence, Italy) using the biuret method and bovine serum albumin at a concentration of 6.02 g/dL as the standard (Biosystems, Barcelona, Spain).

The protein fraction was assessed by an automated system (Selvet24; Seleo Engineering, Naples, Italy) according to the procedures described by the manufacturer. A total of 25 µL of each serum sample was applied to numbered sample wells in cellulose acetate films. Each holder accommodated up to 24 samples. The films were electrophoresed for 28 min at 180 V. After electrophoresis, the films were immediately fixed using an automated system, stained in red stain acid solution for 10 min, and then dried at 37°C. After being destained in acetic acid and dried completely for 15 min, the films were scanned on a densitometer, electrophoretic curves were plotted and the related quantitative specific protein concentrations calculated using software (SelVet 24; Seleo Engineering). All samples were analysed by the same operator, who determined the lines separating fractions in the densimeter tracing. The major protein fractions were divided into albumin, α1-, α2-, β1-, β2- and γ-globulins from the cathode to the anode, according to the recommendation by the manufacturer (5).

Relative protein concentrations within each fraction were determined as the optical absorbance percentage, then the absolute concentration (g/L) and albumin:globulin ratio (A:G) were calculated using the total protein concentration.

Haptoglobin was assessed with an ELISA kit specific for bovine species (PHASE Haptoglobin assay, Tridelta Development, Maynooth, Republic of Ireland), which had sensitivity of 0.005 mg/mL and intra- and inter-assay coefficients of variation of <7% and <6%, respectively. A microtitre plate reader (EZ Read 400 ELISA; Biochrom, Cambridge, United Kingdom) was used to determine haptoglobin levels. All calibrators and samples were run in duplicate, and samples exhibited parallel displacement to the standard curve for ELISA analysis.

Statistical analysis. The obtained data were expressed as mean ± standard error of the mean. Data were normally distributed as determined by the Kolmogorov–Smirnov test (P-value > 0.05). Two-way analysis of variance (ANOVA) for repeated measures was applied to determine the influence of feed supplementation and of time (t0, t1 or t2) on the investigated parameters, followed by Bonferroni's *post hoc* comparison, using Statistica 8 software (Statsoft, now TIBCO, Palo Alto, CA, USA). P-values < 0.05 were considered statistically significant.

Results

All investigated parameters were within the physiological range for the species (17). Two-way ANOVA showed a statistically significant effect of yeast supplementation time on serum total protein, albumin, β_1-globulins, β_2-globulins, γ-globulins and haptoglobin (P-value < 0.001 in all cases). A significant effect of the group was observed on total protein, albumin and γ-globulins (P-value < 0.01 in the three cases) and haptoglobin (P-value < 0.001). In particular, Bonferroni's

post hoc comparison showed an increase of total protein at t2 relative to t0 (−5.44 g/L) and t1 (−5.65 g/L) in the YG, and at t2 *versus* t1 (−1.01 g/L) in the CG. Albumin, β_1-, β_2- and γ-globulins increased at t2 *versus* t0 and t1 in the YG, and they varied in a statistically significant way during the experimental period in the CG.

Table 2 Mean values ± standard error of the mean of studied parameters with statistical differences related to group, measured in the serum of bulls of the control group (CG) without yeast supplementation and the serum of bulls of the group given 5 g of *Saccharomyces cerevisiae* (YG)

Serum parameters	Group	t0	t1	t2
Total protein (g/L)	CG	73.07 ± 0.45	72.40 ± 0.63	74.08 ± 0.40***
	YG	71.47± 0.53	71.26 ± 0.51	76.91 ± 0.47***
Albumin (g/L)	CG	34.46 ± 0.42	31.75 ± 0.34**	33.41 ± 0.41**
	YG	33.84 ± 0.44	33.49 ± 0.34**	35.20 ± 0.40**
α_1-globulins (g/L)	CG	3.68 ± 0.12	3.35 ± 0.09	3.65 ± 0.25
	YG	3.48 ± 0.08	3.19 ± 0.11	3.48 ± 0.08
α_2-globulins (g/L)	CG	8.52 ± 0.16	8.77 ± 0.12	8.47 ± 0.28
	YG	8.66 ± 0.16	8.20 ± 0.18	9.09 ± 0.16
β_1-globulins (g/L)	CG	9.03 ± 0.38	8.14 ± 0.11	9.16 ± 0.21
	YG	8.84 ± 0.25	8.54 ± 0.23	9.82 ± 0.19
β_2-globulins (g/L)	CG	9.78 ± 0.29	9.51 ± 0.28	9.77 ± 0.24
	YG	9.21 ± 0.24	8.79 ± 0.21	10.15 ± 0.22
γ-globulins (g/L)	CG	8.95 ± 0.51	10.89 ± 0.47**	10.95 ± 0.37
	YG	8.54 ± 0.34	9.05 ± 0.32**	10.17 ± 0.36
A:G ratio	CG	0.87 ± 0.02	0.79 ± 0.02***	0.80 ± 0.01
	YG	0.88 ± 0.01	0.89 ± 0.01***	0.83 ± 0.01
Haptoglobin (mg/L)	CG	1.28 ± 0.07	0.75 ± 0.08	0.74 ± 0.06
	YG	1.28 ± 0.09	0.49 ± 0.05*	0.47 ± 0.04*

* – P < 0.05; ** – P < 0.01; *** – P < 0.001 (in Bonferroni's *post hoc* comparison)

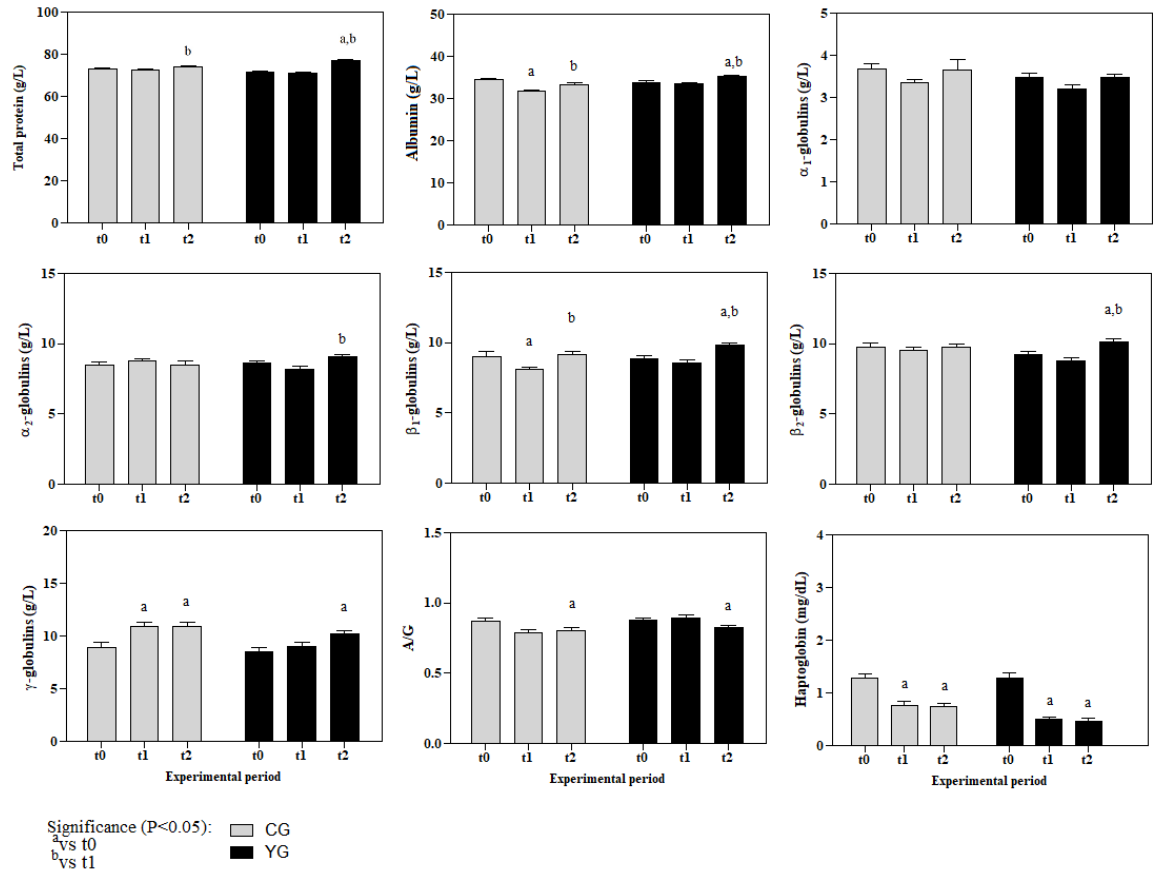

Fig. 1. Mean values ± standard error of the mean of the studied parameters with statistical differences related to time, measured in the serum of bulls of the control group (CG) without yeast supplementation and the serum of bulls of group given 5 g of *Saccharomyces cerevisiae* (YG). Lowercase letters indicate significant difference between time points found by Bonferroni's *post hoc* comparison

Haptoglobin decreased in both groups at t1 and t2 *versus* t0. In Fig. 1 the mean values of the investigated parameters ± the standard error of the mean (SEM) are reported as found in the YG and CG at the different data points, together with the statistical differences due to yeast supplementation time. Bonferroni's *post hoc* comparison showed a significant effect of diet supplementation in total protein at t2, albumin at t1 and t2, γ-globulins at t1, A:G ratio at t1 and haptoglobin at t1 and t2 (Table 2). Total protein was higher by 2.83 g/L in the YG than in the CG. Albumin was higher by approximately 1.75 g/L in the YG than in the CG at t1 and t2. Gamma globulins were lower by 1.84 g/L in the YG than in the CG at t1, and haptoglobin was lower by 0.26 mg/L in the YG than in the CG at t1 and t2. The last measurement providing evidence of the supplementation effect is the A:G ratio, which was higher by 0.1 in the YG than in the CG at t1.

Discussion

One of the principal parameters used for assessing animal nutritional status is total protein. In our study, higher serum total protein values were found in the YG compared to the CG after 40 days of yeast supplementation. The proteins comprising the total are sources of amino acids for muscle protein synthesis and they can be related to muscle mass increase (3). A higher serum concentration of total protein was found in the CG at t2 relative to t1, in the YG at t2 compared to t1 and in the YG at t2 than at t0, and this related to the well-known lower serum total protein concentration in young animals than in adults due to muscle mass increase with age (10). The increased concentration of serum total protein in the YG at the end of the experimental period may indicate the improved nutrient status of the bulls and may have been induced by yeast-supplemented feed intake. In agreement with our results, Adeyemi *et al.* (1) found an increase in serum total protein in a group fed a diet with the *Saccharomyces cerevisiae* supplement over the total in a group fed without yeast diet supplementation. Contrastingly, Li *et al.* (18) found no difference in serum total protein concentration between groups fed with and without yeast diet supplementation, probably because of some benefits of the *Saccharomyces cerevisiae* diet supplement in improving nitrogen utilisation. Increases of serum albumin at t2 and t1 and of A:G ratio values at t1 in the YG were observed. In agreement with our findings, Piccione *et al.* (23) showed increased serum albumin concentration and A:G ratio in a group receiving *Saccharomyces cerevisiae* diet supplementation, as an indicator of yeast-induced energy-balance differences between finishing steers. In contrast, Ma *et al.* (21) found no statistically significant differences in serum albumin concentration between yeast-supplemented and unsupplemented groups. However, increases in the serum total protein and the serum albumin values can be indicators of greater protein outflow from the rumen because of improved ruminal nitrogen utilisation to synthesise microbial protein (29). Also, in the same research of which the findings suggested the higher nitrogen use, Zhang *et al.* (29) found increases in serum total proteins and the serum albumin concentrations in a treated group as consequences of improved ruminal fermentation and feed utilisation efficiency after the administration of yeast supplementation in the diet.

An increase of α_2- and β_2-globulins was found in the YG, while β_1- and γ-globulins increased in both the YG and CG at the end of the experimental period. These findings suggest an activation of the acute-phase response in both groups (1). Burdick Sanchez *et al.* (7) suggested that a calf is better prepared for exposure to a pathogen if its immune system is stimulated by supplementation with a yeast fermentation product. Yeast fermentation products' ability to reduce inflammatory stress has been reported during and after their administration (16). Moreover, a significantly weaker acute-phase response in a group fed a diet with *Saccharomyces cerevisiae* supplementation than in a group fed an unsupplemented diet during the finishing phase was reported by Piccione *et al.* (23). In addition, they found higher serum γ-globulin concentrations in steers fed without yeast diet supplementation than in steers fed with a supplement of *Saccharomyces cerevisiae* in the diet. In contrast, Lipiński *et al*. (19) and Małaczewska *et al.* (22) found higher values of serum γ-globulins in yeast-supplemented groups than in respective control groups of turkeys and of lambs. No effect of yeast supplementation was observed on the α1-globulin serum concentrations.

It is well documented that some changes in diet could result in rumen pH changes, alterations to the microbial ecosystem and the accumulation of large amounts of endotoxin in the organism, and that it is linked to increased peak concentrations of acute-phase proteins, such as haptoglobin. Haptoglobin is one of the most important α2-globulins. It is synthesised by hepatocytes in negligible concentrations in healthy animals and in high amounts in unhealthy ones, and may increase more than 100-fold during the inflammatory and acute-phase response in cattle (28). For this reason, the serum haptoglobin value has been exploited as a useful marker for disease conditions, infections, inflammations, and stressor effects (9). Serum haptoglobin concentration was significantly affected by time of sampling in steers studied by Shen *et al.* (26) whether the animals were given supplements or not, with significantly lower concentration at t2 and t1 with respect to t0 in both examined groups; however, the mean serum haptoglobin concentration remained at all data points within the reference range (serum haptoglobin < 2 mg/dL). No effect of yeast supplementation in the diet of steers on serum haptoglobin concentration was found by Burdick Sanchez *et al.* (7). Other authors nevertheless found a greater haptoglobin concentration in serum from steers provided a yeast-supplemented diet than in serum

from steers provided a standard diet, the higher concentration probably being due to an increase in free circulating haemoglobin (HGB) (25). In a discordant outcome, no change in HGB concentration accompanied the higher serum haptoglobin concentration in bulls treated with *Saccharomyces cerevisiae* fermentation products than in the control bulls noted by Shen *et al.* (26).

Conclusion

It is well established that the use of yeast supplementation in feed has positive effects on the physiology of bulls. Yeast supplementation induced an increase in the serum levels of total protein and albumin as indices of higher body mass gain and a reduced acute-phase response, in association with an increase of the α_2-globulins after 40 days of yeast supplementation. This aspect together with a delayed increase of γ-globulins with respect to the control group and a decrease in serum haptoglobin concentration starting from 20 days of yeast supplementation indicates the reductive effect of *Saccharomyces cerevisiae* on the inflammatory status of young bulls.

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