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Effects of bovine colostrum on performance, survival, and immunoglobulin status of suckling piglets during the first days of life

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ABSTRACT: Supplementation of bovine colostrum (BC) has shown to improve growth performance, intestinal development, and immune response in early-weaned pigs. Little is known about whether BC may have similar effects in neonatal piglets. In the present study, the effect of BC supplementation on mortality, growth performance, and blood parameters (plasma proteins and white blood count) of suckling piglets in the first 10 days of life was investigated under practical conditions with special emphasis on low birth weight piglets. In total, 258 newborn piglets from 30 multiparous sows in a commercial breeding unit were randomly assigned to two different treatment groups. Piglets received either 1 ml of BC orally on days 1–3 of life (group BC, n = 128) or 1 ml of saline (0.9%) (control (CON) group; n = 130). Body weight was measured on days 1, 4, and 10 of life. Blood was collected on days 1 and 4 from 60 piglets per group. No differences in mortality, body weight, and average daily weight gain were observed between treatment groups in days 1–10. However, compared to CON, particularly in low birth weight piglets the administration of BC supported (P < 0.01) their survival. Group BC exhibited lower plasma total protein (P = 0.03) and beta-globulin (P = 0.02) concentrations compared to group CON. In conclusion, BC improved low and normal birth weight piglets' survival during their first 10 days of life. Further research is needed to clarify whether the survival rate is related to earlier gut closure indicated by lower plasma protein levels, which might be beneficial due to a lower uptake of potential antigenic substances.

Keywords: bovine colostrum supplement; growth performance; field trial; nursery pig; survivability

INTRODUCTION

Neonatal mortality is estimated at around 10% in livestock animals (Sangild 2003) and represents one of the major economic losses in pig rearing systems. Pre-weaning mortality mostly occurs during the first days of life (Tuchscherer et al. 2000) and insufficient colostrum intake leads to an increased risk of starvation (Jensen et al. 2001). Sow's colostrum provides piglets with energy for thermoregulation (Le Dividich et al. 2005), immune protection (Klobasa et al. 1981), and promotes intestinal development (Xu et al. 2000). In modern pig production, selection on prolificacy substantially increases numbers of life-born piglets, but also concurs with a greater heterogeneity in birth weights and lower vitality of piglets (Devillers et al. 2011). Since colostrum production is highly variable among sows and independent of litter size (Devillers et al. 2007), ensuring a sufficient colostrum uptake by all piglets emerged to be a major challenge. In addition, colostrum intake depends not only on the amount the sows are capable to produce, but low birth weight and

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vitality also impact piglet's ability to extract colostrum from teats (Hoy et al. 1997). Increasing litter sizes lead to decreased piglets' average birth weights (Devillers et al. 2011); and low birth weight piglets show lower thermoregulatory abilities (Herpin et al. 2002), are less vigorous competing with heavier littermates, and are often crushed by the sow (Rutherford et al. 2013). Additionally, low birth weight is associated with various negative long-term effects like higher disease susceptibility due to an insufficient immunity acquirement as a result of the delayed or restricted suckling after birth (Rutherford et al. 2013).

Bovine colostrum (BC) is a co-product of the dairy industry which contains highly available nutrients and bioactive molecules such as growth and anti-microbial factors (Pakkanen and Aalto 1997) and is particularly promoted by the feed industry to promote weak and low birth weight piglets as well as piglets from big litters. BC comprises high levels of immunoglobulins (Ig) and growth factors such as IGF-1 (de Lange et al. 2010) and contained whey proteins may exert immune-modulatory ability as they were recognized as potent modulators of cellular immune function in livestock species (Boudry et al. 2008b). Beneficial aspects of BC on growth performance, intestinal development, immune parameters, and sanitary status of pigs during the early post-weaning period have been already demonstrated (King et al. 2007; Huguet et al. 2011). Additionally, positive effects of BC on the incidence of necrotizing enterocolitis in preterm piglets were described previously (Bjornvad et al. 2008, Jensen et al. 2013). Although effects of BC have been well described for the post-weaning phase, less information is available for piglet's survival, growth performance, and immune status during the early suckling phase, especially for low birth weight piglets, when the piglet largely relies on the immunity of the sow. Under practical conditions, recommended dosages of commercially available BC supplements for neonatal piglets are much lower than doses tested under experimental conditions (Bridger and Brown 1981; Bjornvad et al. 2008; Jensen et al. 2013). In this study the effect of commercially available BC was tested when provided at a dose typically administered to newborn piglets by farmers. It was hypothesized that already relatively low doses of supplementary BC would promote piglet immunity and growth, thereby would decrease neonatal mortality, especially in less vital and low birth weight piglets. In the present pilot study a commercially available BC product was administered to newborn piglets under field conditions to assess potential effects onto piglet survival rates, growth rates, occurrence of diarrhea, and changes in white blood count and Ig in low and normal birth weight piglets.

MATERIAL AND METHODS

The experimental protocol used in this study has been approved by the German State Agency for Agriculture, Food Safety and Fishery Mecklenburg-Western Pomerania (Certificate No. 7221.3-1.1-081/12).

Animals and experimental design. A total of 258 newborn piglets ((Landrace × Large White) × Piétrain) from 30 multiparous sows of a commercial pig breeding farm in Mecklenburg-Western Pomerania, Germany, were included in this study. Sows were housed in groups during mating and gestation and were moved to farrowing pens (with individual farrowing crates) seven days prior to the expected farrowing date. Sows were fed commercial gestation and lactation diets. Water was provided via nipple drinkers *ad libitum*. On day 114 of gestation, sows were injected intramuscularly with 0.7 ml of a synthetic prostaglandin analogue (Estrumate 250 µg/ml; Intervet GmbH, Unterschleissheim, Germany) to induce parturition within the next 24 h. Farrowing pens were diffusely ventilated with a room temperature of 18–21°C. Heating plates were provided for the piglets at one side of the pen next to the sow. Parturitions were monitored, but interference was kept at a minimum. Piglet performance was followed from birth until day 10 of life. Immediately after birth, all piglets per litter were weighed and 8 ± 1.74 (SD) piglets per litter, depending on litter size, were randomly assigned to one of the two treatment groups and individually marked with ear tags. Piglets in group BC (n = 128) received 1 ml of bovine colostrum preparation orally (Vetilan COLOSTRUM BOOST; Veracus GmbH, Bremerhaven, Germany) on three consecutive days (1 ml/piglet on days 1, 2, and 3 of life), while piglets in group CON (n = 130) served as negative control receiving sterile saline orally (1 ml 0.9% NaCl/piglet on days 1, 2, and 3 of life). Vetilan COLOSTRUM BOOST is a product licensed to be fed to weak and small piglets immediately after birth and, if needed, every 24 h thereafter (1–2 ml per piglet). According to the manufacturer's specifica-

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tion, it consists of 100% sterile filtered, de-fatted, and de-caseinated bovine colostrum containing more than 20% immunoglobulins. Piglets were not cross-fostered so that litters were kept together regardless of their size. All piglets were allowed to suckle their dams at any time of the day and received an injection of iron intramuscularly (1 ml/pig Ursoferran 200 mg/ml; MEDISTAR Arzneimittelvertrieb GmbH, Bernburg, Germany) on day 4 of life. Body weight of all piglets was recorded on days 1, 4, and 10 of life. Blood samples (groups BC and CON: n = 60; 1–2 ml blood/piglet) of the two heaviest piglets of the same litter from each treatment group were taken by puncture of the anterior vena cava on days 1 and 4 of life. The samples were collected into EDTA tubes (KABE®, Nuembrecht-Elsenroth, Germany). The heaviest piglets were selected for blood collection to minimize the possibility of missing samples on day 4 due to animal losses. Throughout the experimental period (days 1–10), piglets were daily examined for signs of diarrhea and the number of dead piglets was recorded.

Chemical analysis. Cytological and biochemical blood analyses were performed according to routine methods. Complete blood counts including white blood count (WBC) were determined in both groups of piglets in an automatic cell counter (MEK-6108G; Nihon Kohden Europe GmbH, Rosbach, Germany). Differential cell counts were determined by counting of 200 cells in blood smears after Pappenheim staining. Total protein concentration was determined photometrically by biurette method. Blood samples were centrifuged at 2000 *g* for 15 min and plasma was stored at –20°C until further analysis. Plasma protein contents (total protein, albumin, alpha-1-, alpha-2-, beta-, and gamma-globulin) were determined by plotting the EDTA plasma onto a cellulose acetate film (Elphor, Sarstedt AG & Co., Nuembrecht-Rommelsdorf, Germany) and proteins were separated in an electric field according to their size and charge by electrophoresis using an integrated scanning system Elphoscan Mini Plus (Sarstedt AG & Co.).

Statistical analyses. To compare differences between treatment groups, data were subjected to ANOVA using the PROC MIXED of SAS (Statistical Analysis System, Version 9.2, 2009).

To investigate the effect of BC supplementation, fixed effects in the model included the main effects of BC supplementation and sex as well as the random effect of sow, considering the pig as the experimental unit. The day of the experiment when a piglet died was used to estimate the piglet's survival time. To estimate whether plasma parameters were different at the two sampling days, data collected on the same pig and two sampling days were considered as spatial repeated measures. The covariance structure was modelled separately according

Table 1. Effect of bovine colostrum supplementation on survival time and growth performance of suckling piglets in days 1–10 of life

Parameter	Group BC		Group CON		Pooled SEM	*P*-value
	mean	*n*	mean	*n*		
Days of life of piglets that died during days 1–10						
All piglets	5.7[a]	15	2.8[b]	12	0.53	0.002
25% low birth weight piglets	5.7[a]	15	2.4[b]	9	8.90	0.001
Initial BW (kg)						
All piglets	1.4	118	1.4	128	0.04	0.726
25% low birth weight piglets	0.9	32	0.9	27	0.03	0.642
Final BW (kg)						
All piglets	3.0	103	3.0	115	0.09	0.449
25% low birth weight piglets	2.5	16	2.3	26	0.11	0.484
ADG days 1–10 (g/day)						
All piglets	153.7	103	154.8	115	6.53	0.904
25% low birth weight piglets	110.1	16	120.6	26	17.91	0.685

group BC = Vetilan COLOSTRUM BOOST administration on days 1–3 of life, group CON = control, with 0.9% NaCl administration on days 1–3 of life, BW = body weight, ADG = average daily gain

data are presented as Least Squares Means ± SEM

[a,b]values with different superscripts differ significantly at $P < 0.05$

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to the smallest values of the fit statistics based on the Bayesian information criteria (BIC). Means were reported as Least Squares Means ± standard error of the mean (SEM) with $P \leq 0.05$ defined as significant and $0.05 < P \leq 0.10$ as trends. Degrees of freedom were approximated using Kenward-Rogers method (ddfm = kr). To investigate whether BC supplementation affected particularly weak piglets, 25% of piglets with the lowest birth weight of each treatment group were analyzed separately. Pearson's correlation coefficients among variables of blood parameters and body weights at birth and at days 4 and 10 of life were determined using PROC CORR.

RESULTS

The present sows delivered on average 12.93 ± 2.76 (SD) piglets per litter with 34 out of 258 (i.e. 13.2%) piglets weighing less than 1.0 kg at birth. Litters of 16 or more siblings (two sows with $n = 16$ piglets, three sows with $n = 17$, $n = 18$, and $n = 20$ piglets each) existed and piglets were not cross-fostered.

Mortality. During their first 10 days of life, 28 out of 246 (11.4%) study piglets died due to different reasons. Mortality was not different between treatment groups (group BC, $n = 15$ (12.7%), group CON, $n = 13$ (10.2%); $P = 0.72$, SEM = 0.13). Days of life of piglets of the two different groups that died in the experimental period are displayed in Table 1. With BC intake, the piglets that died within the experimental period were by 3 days longer alive than the piglets of CON group ($P < 0.01$). In piglets with a very low birth weight, BC supplementation significantly prolonged their life during the first 10 days of life compared to piglets of CON group (Table 1).

Table 2. Hematological parameters of groups BC and CON piglets at days 1 and 4 of life

Parameter[2]	Group BC	Group CON	Pooled SEM	*P*-value
WBC (10^9/l)				
Day 1	12.3	13.0	0.80	0.239
Day 4	10.5^{A}	9.7^{B}	0.38	0.069

group BC = Vetilan COLOSTRUM BOOST administration on days 1–3 of life ($n = 54$), group CON = control, with 0.9% NaCl administration on days 1–3 of life ($n = 54$), WBC = white blood count

data are presented as Least Squares Means ± pooled SEM

^{A,B}values with different superscripts demonstrate trends at $P < 0.1 > 0.05$

Animal growth performance and sanitary status. Piglet body weight did not differ between the two treatment groups on days 1, 4, and 10 of life ($P \geq 0.45$). Likewise, average daily gain (ADG) was similar between treatments, being 153.7 g/day ± 6.69 (SEM) and 154.8 g/day ± 6.37 (SEM) for group BC and CON ($P = 0.90$), respectively (Table 1). Also, the piglets by 25% lower in birth weight had similar body weight ($P > 0.48$) and ADG ($P = 0.69$) in both treatment groups. Regardless of the treatment group, around 30% of the piglets showed mild to severe diarrhea during the experimental period (days 1–10 of life).

Hematological parameters. Blood samples were collected from piglets in groups BC ($n = 60$) and CON ($n = 60$) on day 1 and from the same piglets on day 4 of life with 54 out of 60 suitable sera for analysis in both treatment groups. Mean WBC values of both treatment groups are presented in Table 2. In piglets which received BC a tendency

Table 3. Serum proteins of group BC and CON piglets at days 1 and 4 of life

Parameter (g/l)	Group BC	Group CON	Pooled SEM	*P*-value
Total protein				
Day 1	52.7	53.5	3.00	0.651
Day 4	58.7^{a}	62.4^{b}	1.39	0.032
Albumin				
Day 1	6.7	6.7	0.32	0.650
Day 4	13.6^{A}	14.3^{B}	0.41	0.085
Alpha-1				
Day 1	2.0	2.0	0.11	0.600
Day 4	1.8^{A}	2.0^{B}	0.06	0.076
Alpha-2				
Day 1	6.0	6.2	0.44	0.681
Day 4	8.8	9.0	0.28	0.570
Beta				
Day 1	7.4	8.0	0.41	0.208
Day 4	11.6^{a}	12.6^{b}	0.47	0.018
Gamma				
Day 1	30.4	30.6	2.77	0.913
Day 4	22.9	24.5	0.94	0.155

group BC = Vetilan COLOSTRUM BOOST administration on days 1–3 of life ($n = 55$), group CON = control, with 0.9% NaCl administration on days 1–3 of life ($n = 57$)

data are presented as Least Squares Means ± pooled SEM

^{a,b}values with differnet superscripts differ significantly at $P < 0.05$

^{A,B}values with different superscripts demonstrate trends at $P < 0.1 > 0.05$

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towards an increase in WBC ($P = 0.07$) compared to piglets of group CON on day 4 (Table 2) was seen.

Plasma proteins. Total protein, albumin, alpha-1-, alpha-2-, beta-, and gamma-globulin were determined in 55 and 57 blood samples collected from 60 piglets in groups BC and CON on days 1 and 4 of life, respectively. From days 1 to 4, total protein, albumin, alpha-2-, beta-globulin increased and alpha-1- and gamma-globulin decreased for both groups. Group BC piglets had lower total protein ($P = 0.03$) and beta-globulin ($P = 0.02$) concentrations and, as trend, lower albumin ($P = 0.09$) and alpha-1 ($P = 0.08$) concentrations compared to group CON piglets on day 4 of life (Table 3). In both groups (BC and CON), a significant correlation was found between the protein concentration and the concentration of gamma-globulins at birth ($r = 0.96$, $P < 0.001$) and between body weight (BW) on day 10 and the protein concentration at birth (group BC: $r = 0.45$, $P < 0.001$, group CON: $r = 0.27$, $P = 0.04$). In contrast, significant negative correlations ($r = -0.4$– -0.29, $P < 0.05$) were found for alpha-1-globulin concentrations on days 1 and 4 with BW on days 4 and 10, respectively.

DISCUSSION

In modern pig production, numerous offspring is an economic trait leading to increased numbers of piglets born per litter over the last decades. Hyper-prolific sows with more than 16 live-born piglets per litter often have a greater heterogeneity in piglet birth weight and a higher number of weak piglets at birth (Devillers et al. 2011), which was confirmed by the litter size and number of low weight piglets in the present study. It is widely recognized that pre-weaning mortality is positively correlated with litter size (Dyck and Swierstra 1987) and still remains an unsolved problem. Low birth weight is unanimously associated with reduced chances of survival (Cabrera et al. 2012) and thus increasing survival rates of vulnerable piglets is one of the biggest challenges. As causes for pre-weaning deaths are various and low colostrum intake is probably the most influential one, in this study, a commercially available BC product at a dosage typically used at pig breeding farms was supplemented to newborn piglets with special emphasis on low birth weights. Weak piglets, among those very low birth weight piglets, generally have a lower colostrum intake (Devillers et al. 2007) and early assistance is particularly beneficial to promote their survival. Here, we could show that piglets, including low birth weight piglets, have benefited from the extra BC in terms of a longer survival indicating that their colostrum intake might have been insufficient or that the amount of bioactive substances ingested with sow colostrum might have been too low to meet their special nutrient needs. According to the product declaration, the recommended BC dosage is 1–2 ml per piglet, which is much less than previously reported BC ranges in research studies (Bridger and Brown 1981; Gomez et al. 1998; Moller et al. 2011; Huang et al. 2012). Taking into account, that sow-reared piglets consume on average 250–300 g/kg birth weight colostrum during the first 24 h after birth (Quesnel et al. 2012), the recommended administration level of BC was most likely not high enough to clearly enhance piglet growth and survival. Several colostrum supplements, bovine and swine, are marketed, but scientific evidence on their effectiveness is sparse and natural colostrum seems to be superior to enriched formulae (Baxter et al. 2013). It was found that neither supplementation with 15 ml of sow colostrum (combined with cross-fostering) (Muns et al. 2014), nor 2 × 25 ml of sow colostrum (without continued aftercare) did improve piglets' survivability (Muller et al. 2012) illustrating the multifactorial nature of piglet mortality. By giving piglets 12 ml of first milking BC, a reduction in pre-weaning mortality could be demonstrated by White et al. (1996). However, in the study of White et al. (1996) the BC supplementation was only one part of a multifaceted farrowing management protocol (White et al. 1996). In our study, a single dose of the tested BC on day 1 of life did not result in any beneficial effects on mortality, body weight, average daily weight gain, nor viability (data not shown), but despite the relatively small amount of BC used, repeated supplementation on days 1–3 of life did influence piglets' survivability (normal and low birth weights). The observed beneficial effects of BC may have been therefore cumulative in piglets and it needs to be verified whether a higher dosage or a longer administration time of the commercial BC product might prevent the death of weak newborn piglets during the suckling phase. Overall, the present mortality rate of about 12% was in the range of pre-weaning mortality in piglets previously described (Devillers et al. 2011) and was not influenced by BC administration.

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Compared with high birth weight littermates, light born piglets are disadvantaged in extracting colostrum from teats (Hoy et al. 1997). Nonetheless, sow's colostrum is crucial for piglet's energy supply needed for thermoregulation (Le Dividich et al. 2005) and glucose homeostasis (Herpin et al. 1992) as piglets are born without fat stores (Le Dividich et al. 1991). The nutritional importance of colostrum for the survival and body growth of newborn pigs is e.g. indicated by the high digestibility of proteins in porcine colostrum of up to 100% (Lin et al. 2009). Jensen et al. (2001) demonstrated a considerable uptake of bovine macromolecules from cow's colostrum in newborn piglets during the first 12 h after birth (Jensen et al. 2001). Although BC intake was comparatively small in the present study, piglets with very low birth weight may have profited most from the extra energy intake by BC administration enhancing their suckling capacity, which might have been indicated by the longer survival. However, in considering the daily administration dose, it may be rather assumed that the nutritional effect of BC may be of lower importance in the present field study, particularly because piglets could suckle freely during the experimental period. Therefore, it was not surprising that BW and ADG of normal and low birth weight piglets were similar among treatment groups.

Several studies showed a direct correlation between newborn mortality and plasma IgG concentrations (Klobasa et al. 1981; Cabrera et al. 2012). In the present study, the positive correlation between first-day protein concentrations and BW on day 10 implicates the necessity for uptake of energy and maternal proteins as the basic prerequisite for piglet vitality and growth-performance in its future life. Regarding the plasma protein content, a similar increase in total protein, albumin, alpha-2-, and beta-globulin was found from day 1 to 4 of life for all piglets, independently of the BC administration, surpassing the essential threshold level of IgG of 10 mg/dl previously suggested (Machado-Neto et al. 1987; Carbrera et al. 2012). Due to the epitheliochorial placentation, piglets are born without systemic immunity in form of Ig and disease resistance mainly relies on an adequate colostrum and IgG uptake (Porter 1969; Coalson and Lecce 1973; Klobasa et al. 1981; Sangild 2003). Colostrum and milk offer both, high amounts of maternal cells (B and T cells) and other substances e.g. cytokines modulating offspring's immunity (Wagstrom et al. 2000). Typically, neonatal piglets preferentially absorb porcine IgG relative to bovine IgG (Jensen et al. 2001). Although absorption of bovine Ig cannot provide a noteworthy passive immunity against pig pathogens, piglet survival has been reported to benefit from bovine Ig supplementation compared to being fed milk replacer completely devoid of Ig (Gomez et al. 1998). These results suggest that colostrum *per se* may contain nutrients and bioactive factors positively affecting disease resistance in a species-independent manner (Sangild 2003), which may be related to innate immunity receptor compatibilities and antibody specificity targeted at pathogen associated molecular patterns covering also microbe associated molecular patterns of sows' indigenous microbiota, respectively. Interestingly, in the present study, a decrease in plasma protein levels (total protein, albumin, alpha-1- and beta-globulin) was found in BC supplemented piglets compared to control piglets. Because the supplemented BC was only a small part of the overall colostrum consumption of the piglet, the reason for the lower protein levels with BC supplementation might be related to a reduction in gut permeability triggered by the extra BC consumption and thus might hint at an earlier gut closure of piglets receiving BC compared to the control piglets as previously demonstrated by Jensen et al. (2001). Albumin is one of the major porcine colostral proteins (Gallagher et al. 1997) and the absorption of bovine serum albumin by neonatal pigs has been previously described (Westrom et al. 1984; Huang et al. 2012). Plasma beta-globulin contains, among others, the acute-phase-protein (APP) C-reactive protein, an unspecific marker for inflammation (Kushner 1982). Likewise, alpha-1 globulin consists of APPs (Kushner 1982) and its negative correlation with piglets' BW, together with the lower plasma beta-globulin concentration in piglets receiving BC, might support our assumption that BC supplementation might affect gut permeability leading to an accelerated gut closure, thereby reducing the risk of pathogen invasion. BC supplementation has been reported to improve sanitary status in post-weaning (Huguet et al. 2006, 2011; Boudry et al. 2008a, 2010) and preterm (Moller et al. 2011) piglets. Here, we did not find an effect on the prevalence of diarrhea occurring in both treatment groups which may probably be associated with the relatively low BC dosage and

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frequency. The improved WBC levels in group BC piglets compared to group CON piglets might be related to the extra micronutrients (e.g. Fe) and bioactive substances of the BC product used.

Although our experimental period of 10 days was relatively short and BC dosage used comparatively small, effects on piglets' blood parameters and survival could be demonstrated positively affecting piglet survivability, particularly in low birth weight piglets. However, more research is needed to elucidate the mode of action of BC supplementation at gut level in newborn pigs in the first 4 days of life.

CONCLUSION

In conclusion, data from this pilot field study suggest that newborn piglets' early survival might be positively influenced by the BC product used, particularly in piglets of low birth weight. BC ingestion reduced plasma levels of proteins compared to the control indicating an earlier intestinal closure. Further long-lasting studies in newborn piglets under experimental conditions, at least until weaning, are needed to clarify the exact mode of action of BC on (low birth weight) piglet's survival and whether stronger effects may be achieved by a more frequent BC administration.

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