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# Milk consumption monitoring as a farmer friendly indicator for advanced treatment in limited fed calves with neonatal diarrhoea syndrome

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**Abstract:** The purpose of this study was to investigate whether milk consumption (MC) could be used as a simple farmer-friendly indicator for providing advanced treatment to limited fed diarrhoeic neonatal calves. Complementarily, it was evaluated whether the standard indications for veterinary care (severe dehydration and/or acidosis-septicaemia) are associated with different patterns in MC. The MC and health records of 103 calves with diarrhoeathat were fed a milk replacer at the volume of 10% of their body weight were used in the study. The MC reduction rate (MCRR) was calculated after each feeding ($MC_t$) during the diarrhoea course, based on the MC prior to the diarrhoea onset for each calf ($MC_0$) using the formula $MCRR = 100 \times (MC_0 - MC_t)/MC_0$. The calves were assigned into the ST group ($n = 58$) if they only received the standard treatment (oral rehydration solutions between milk feedings) until recovery, and into the advanced treatment (VT) group ($n = 45$) if they needed advanced treatment (*i.v.* fluids ± antibiotics) directly or after the standard treatment. The calves in the VT group that only had dehydration, were further assigned into the DH subgroup ($n = 22$) and those with signs of acidosis-septicaemia with or without dehydration were assigned into the ASD subgroup ($n = 23$). The MC was practically stable in the ST group throughout the diarrhoea course. In the VT group, the MC was significantly reduced during the last 36 hours prior to the advanced treatment administration. This reduction was significantly higher in the ASD subgroup than the DH subgroup. The MCRR on the last meal prior to treatment administration was proven to be a very reliable indicator for the detection of diarrhoeic calves needing advanced treatment (cut-off: ≥ 24.5%; sensitivity: 95.6%; specificity: 98.7%) and of those with acidosis-septicaemia (cut-off: ≥ 29.6%; sensitivity: 91.7%; specificity: 99.1%). The results of the study show thatfarmers employing this feeding regimen should seek veterinary assistance when the milk consumption of calves is reduced by ≥ 24.5%.

**Keywords:** milk intake monitoring; calf scours; intensive therapy; farmer guidelines

Diarrhoea syndrome is the most common cause of illness and mortality in neonatal calves (Uhde et al. 2008; Izzo et al. 2011). Enterotoxigenic *Escherichia coli* K99/F5 (ETEC), rotavirus A, Bovine coronavirus and *Cryptosporidium spp.* are the most common infectious agents implicated with the aeti-

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ology of this syndrome (Uhde et al. 2008; Izzo et al. 2011; Cho and Yoon 2014; Katsoulos et al. 2017). Co-infections with more than one of these pathogens are frequently detected in clinical practice and attempts to distinguish between the agents are usually unrewarding (Taylor et al. 2017). Regardless of the causative agents, diarrhoea, if left untreated, can lead to dehydration, electrolytic imbalances, acidosis and possibly septicaemia due to the secondary bacterial overgrowth in the small intestine (Berchtold 2009; Taylor et al. 2017).

Early detection of diarrhoeic calves and the prompt intervention increases the success rate of the treatment. Nowadays, it is common practice for the initial diagnosis and treatment of neonatal calf diarrhoea to be predominantly carried out by primary producers (e.g., farmers or farm managers), who utilise oral rehydration and buffering solutions as the first inexpensive attempt to address diarrhoea (Sayers et al. 2016).

However, in the course of the disease, some calves will need intravenous (*i.v.*) fluid therapy to correct severe dehydration, acidosis and septicaemia (Naylor et al. 2006; Berchtold 2009; Meganck et al. 2014).

Therefore, it would be of great value for the success of treatment if the producers had a quantitative indicator available to use for the early detection of the symptoms associated with these complications to enable early veterinary assistance to be sought.

Although it is well documented that illness is associated with alterations in the milk feeding behaviour in calves (Svensson and Jensen 2007; Borderas et al. 2009; Sutherland et al. 2018), there is no relevant reference in the available literature suggesting that milk consumption (MC) could be used as a tool for determining when to provide treatment. In addition, evidence in literature suggests that *ad libitum* fed sick calves have different feeding behaviour than sick calves with a low milk allowance (Borderas et al. 2009).

The purpose of the present study was to investigate, under field conditions, whether the MC could be used as a farmer friendly indicator for providing advanced treatment to limited fed diarrheic neonatal calves.

A complementary objective was to evaluate if the different indications for veterinary care, dehydration and/or acidosis-septicaemia, are associated with different patterns in the MC.

## MATERIAL AND METHODS

### Farms and feeding practices

The study was conducted on two Greek dairy farms (Farm A and Farm B) from September 2016 to February 2018. The selection criteria for these farms were:

1. A high incidence rate of the diarrhoea syndrome;
2. the identical management practices concerning calves' feeding management;
3. the potentiality for the daily monitoring the calves' MC;
4. the keeping of health records;
5. the willingness of the farmers to participate in the study.

Newborn calves in Farm A were housed in concrete floor group pens of 12 $m^2$ for 12 calves whereas those in Farm B were housed in individual concrete floor pens of 2 $m^2$. Each pen in Farm A was equipped with concrete feed troughs on the external side and water bowls mounted inside the pens. In Farm B, the feed and water buckets were secured outside the pen. Long stem wheat straw was used for the bedding in both farms; the wet bedding was removed daily and was totally replaced twice a week. In winter, oil heaters were used as aheat supplementation when the ambient temperature was below 4 °C.

On both farms, the newborn calves were bottle-fed fresh colostrum obtained from their respective mothers, at a volume of 2 l within 2 h after birth and another 2 l after 6 hours. At 2 and 3 days of age, all the calves were fed colostrum/milk from their respective dams, at 10% of their bodyweight offered at 12 h intervals. At ≥ 4 days of age, the calves were provided a milk replacer (Bovilac; Pharmacell, Ltd, Diavata Thessaloniki, Greece; Table 1) at 10% of their bodyweight after dilution (125 g/l). The milk replacer was offered at 39 °C twice a day in a bucket. The MC of each calf was evaluated after each feeding by measuring the volume of the residual milk. Along with the milk, fresh water was offered *ad libitum*, at all times and was replaced daily. Approximately 250 g of calf starter per day was also offered in a shallow bucket beginning at 5 days of age. The amount of the calf starter was increased gradually, as the calves started consuming all of the feed. All the buckets were emptied and refreshed twice per day with new clean feed.

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Table 1. The nutrient and ingredient analysis of the milk replacer used in both farms of the experiment

| Milk replacer nutrient analysis (on dry matter basis) | |
|---|---|
| Crude protein | 22.00 % |
| Crude fat | 16.00 % |
| Crude fibre | 1.10 % |
| Calcium | 0.65 % |
| Phosphorus | 0.45 % |
| Sodium | 0.25 % |
| Ash | 6.50 % |
| Vitamin A (3a672a-Retinyl acetate) | 25 000 IU/kg |
| Vitamin D3 (E671-Cholecalciferol) | 5 000 IU/kg |
| Cupric sulphate pentahydrate (E4-Copper) | 7.00 mg/kg |
| Ferrous sulphate monohydrate (E1-Iron) | 50.00 mg/kg |
| Calcium iodate anhydrous (E2-Iodine) | 0.15 mg/kg |
| Sodium selenite (E8-Selenium) | 0.10 mg/kg |
| Manganous sulphate monohydrate (E5-Manganese) | 35.00 mg/kg |
| Cobalt sulphate (E3-Cobalt) | 0.60 mg/kg |
| Zinc sulphate monohydrate (E6-Zinc) | 60.00 mg/kg |
| Butylated hydroxytoluene (E321) | 30.00 mg/kg |

Ingredients: Whey, wheat gluten, palm oil, lactose free whey, coconut oil, wheat flour, dextrose, calcium carbonate, antioxidant, vitamin and mineral premix

## Clinical evaluation and treatment of diarrhoeic calves

The faecal consistency was evaluated and recorded every day for each calf after the morning feeding by the farm staff using a three-point scale: 1 = normal, 2 = intermediate and 3 = watery faeces (Katsoulos et al. 2017). Calves with faecal scores (FS) ≥ 2 were considered as diarrhoeic. All diarrheic calves were clinically examined every day after the morning feeding from the onset of the diarrhoea until recovery, when FS = 1, by the same veterinarian on each farm (PDK for Farm A and AD for Farm B) who was unaware of the MC. The degree of dehydration, determined according to Naylor (1987) and Constable et al. (1998), the ability of the calf to stand and suckle as well as the severity of the depression and the rectal temperature were the clinical signs that were taken into account for selecting the appropriate treatment regime as suggested by Berchtold (2009). The treatments used and the criteria for the selection are presented in Table 2. A standard treatment was

Table 2. The criteria used for the administration of the standard or advanced treatment in the diarrhoeic calves

| Standard treatment | Advanced treatment |
|---|---|
| Dehydration < 8% | dehydration ≥ 8% |
| Calf standing and strong | calf standing but weak<br>calf unable to stand |
| Strong suckling reflex | weak or absent suckling reflex |
| Normal rectal temperature | abnormal rectal temperature |

applied to all calves that were not considerably dehydrated (< 8%) and had no signs of acidosis-septicaemia (weakness or the inability to stand, a weak or absent suckling reflex, a fever) from the first day of the diarrhoea until recovery; based on the dehydration status, it consisted of offering one or two extra meals of 2 l per day of an oral electrolyte solution (Elektrofit; Over Vet®, Lask, Poland) between the milk feedings. The advanced treatment consisted of an *i.v.* fluid therapy with or without antibiotic administration and was offered to the calves with significant dehydration (≥ 8%) and/or signs of acidosis-septicaemia. The clinical signs and the applied treatment each day were recorded as part of the health records of each calf.

## Study design

The MC and health records of 103 diarrhoeic Holstein calves (66 from Farm A and 37 from Farm B) with an average birth weight of 43.6 kg (SD: 3.1 kg) were used for the purpose of the study. The milk consumption reduction rate (MCRR) was determined for each diarrhoeic calf at each feeding during the diarrhoea course using the following formulae:

$$MCRR = 100 \times (MC_0 - MC_t)/MC_0 \quad (1)$$

where:

$MC_0$ – average $MC$ of the last two meals prior to the onset of diarrhoea;

$MC_t$ – $MC$ recorded at each time point (12 h intervals).

Based on the health records, the diarrhoeic calves were divided into two groups according to the treatment they received; the ST group ($n$ = 58) consisted of calves that only received standard treatment (ST) since the first day of diarrhoea detection until recovery and the VT group ($n$ = 45) consisted

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of those that, apart from the standard treatment, needed advanced treatment (VT) some time during the course of the disease. The calves of the VT group were further divided into two subgroups according to the indications for advanced treatment; the calves that only had dehydration (DH) were assigned into the DH subgroup ($n$ = 22) and those that had signs of acidosis-septicaemia (ASD) (weakness or the inability to stand, a weak or absent suckling reflex, a fever) with or without dehydration into the ASD subgroup ($n$ = 23).

### Ethics

All the procedures were carried out according to the national law and the guidelines of our Institutional Animal Care and Use Committees and followed the ethical standards in the Helsinki Declaration of 1975, as revised in 2000. A formal approval from the Ethical Committees of our Institutions was not required, since no sample was acquired for diagnostic or monitoring purposes and only typical clinical procedures were performed under informed farmer consent.

### Data analysis

Prior to the onset of the experiment, the minimum required total sample size was calculated using the Hotelling-Lawley Trace test at the GLIMMPSE software (http://glimmpse.samplesizeshop.org/). The desired power was set at 0.8 and the type I error rate at 0.05. The desired detectable differences of the MC were 10% and 15% for the DH and ASD groups, respectively, compared to the ST group. So, the input means were 2 l, 1.8 l and 1.7 l for the ST, DH and ASD groups, respectively. The expected relative group size was set at 2 : 1 : 1 and the standard deviation at 0.2. The mean scale factor was set at 1 and the variability scale factor at 2.

Data were analysed using the statistical program SPSS® Statistics v25 (IBM, Chicago, IL, USA). The interrater reliability between the two veterinarians concerning the necessity of the advanced treatment was tested with a kappa coefficient analysis in 15 diarrhoeic calves prior to the onset of the study. The normality of data distribution was assessed with the Kolmogorov-Smirnov test and the homogeneity of the variances was evaluated with the Levene test. A one-way ANOVA (analysis of variance) was used for the comparison of the age of the diarrhoea onset among the groups. In the ST group, a univariate ANOVA with repeated measures was run to evaluate the effect of the time relative to the onset of the diarrhoea (time), of the Farm and their interactions (time × farm) on the MCRR. The same model was also used in the VT group for the determination of the effect of the time relative to the administration of the advanced treatment (time), of the subgroup (DH and ASD) and of the farm as well as their interactions on the MCRR. In all the models, the main effects of the evaluated factors were tested with the Bonferroni test. In order to investigate whether the MC could be used as an indicator for the detection of animals that need the advanced treatment and those that have acidosis-septicaemia, the optimal cut-off value of the MCRR, yielding the highest diagnostic sensitivity (DSn) and specificity (DSp), was determined by a receiver operating characteristic (ROC) analysis. A value of $P < 0.05$ was considered significant in all the comparisons.

## RESULTS

This study investigated whether the MC under the described conditions could be used as a practical indicator for providing advanced treatment to diarrhoeic neonatal calves. It was also investigated whether the standard indications for veterinary care (severe dehydration and/or acidosis-septicaemia) are associated with different patterns in the MC. The calves were not offered milk *ad libitum*, but had a low milk allowance at the rate of 10% per day divided into two meals. Despite that this practice may lead to sub-optimal supply of nutrients for their growth and development (Jasper and Weary 2002; Van Amburgh et al. 2011), at least until the calves start consuming a significant amount of starter feed, it still remains the common feeding practice for dairy calves in Greece and especially in farms where the use of automatic milk feeders is not an option. Acidosis and septicaemia were included in the same treatment category (ASD), given that they share common symptoms and are practically indistinguishable at a clinical level (Lorenz 2009; Pardon and Deprez 2018). It was not attempted to distinguish between the causative infectious agents implicated in each diarrhoea case,

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as this was not included in the objectives of this study. The interrater reliability of the two veterinarians concerning the decision for the advanced treatment administration was strong (k = 0.857, SE = 0.137; $P = 0.001$) according to the classification of McHugh (2012), and thus the calves in both farms were treated in the same way.

The results of sample size analysis revealed that a minimum of 52 calves (26 for the ST, 13 for the DH and 13 for the ASD groups) was required (Power = 0.808) for the purpose of this study. So, the number of calves used (103) was considered as sufficient for acquiring accurate results. Based on their health records, 58 calves were assigned to the ST group and 45 to the VT group. Twenty-two calves from the latter were assigned into the DH subgroup and 23 into the ASD subgroup.

The effect of the factor "time" on the MC was analysed in the different models in the ST and VT groups because, in the last group, the animals received advanced treatment on different days relative to the onset of diarrhoea. Therefore, using the factor "time relative to the treatment" was considered more appropriate than using "time relative to the onset of the diarrhoea" for the objectives of this study.

In the ST group, the average duration of the diarrhoea syndrome was 3.52 days (SD: 1.59 days) and was not significantly different among the farms (mean ± SEM: 3.45 ± 0.36 days and 3.55 ± 0.26 days for Farms A and B, respectively; $P > 0.05$). The repeated measures analysis revealed that the average MC was slightly reduced in this group (mean ± SEM of MCRR: 2.95 ± 3.03%) and was not significantly affected either by the duration of the disease course ($P > 0.05$), by the farm (mean ± SE of MCRR: 2.83 ± 0.36% and 3.26 ± 0.45% days for Farms A and B, respectively) or the interaction time × group ($P > 0.05$). This indicates that the MC at the limited fed calves remained practically unaffected in the uncomplicated cases of the neonatal diarrhoea syndrome and that the described administration of the oral rehydration solution as an extra meal between milk feedings was not negatively associated with the MC.

In contrast, the MC declined during the last days immediately prior to the advanced treatment administration in the VT group. The MCRR in this group was significantly affected by the time relative to the treatment ($P < 0.05$; Figure 1) without, however, being significantly affected by the farm (mean ± SE: 8.99 ± 0.91% and 8.29 ± 0.99% for Farms A and B, respectively; $P > 0.05$) or the interaction time × farm ($P > 0.05$). The MCRR was significantly increased every 12 h during the last 36 h prior to the advanced treatment administration (Figure 1). The analysis of the data of the VT group, taking the indications for treatment into consideration, revealed that the MCRR followed the same pattern in the DH and ASD subgroups; significant differences were also identified at the same time points. Although the MCRR was not significantly different either among the subgroups ($P > 0.05$) or the farms ($P > 0.05$), the interaction subgroup × time had a significant effect ($P < 0.05$). This is attributed to the significantly higher MCRR in the ASD group compared to the DH group at the day of the advanced treatment administration (time point 0; Figure 2). These results indicate that the MC dropped rapidly, within 24 h, in the calves that needed veterinary treatment and that this drop was even higher in the calves with acidosis-septicaemia. Furthermore, they provide evidence for further evaluation of MC monitoring as an indicator for veterinary assistance in diarrhoeic calves.

The ROC analysis revealed that using the cut-off point of ≥ 24.5% for the MCRR, the MC on the last meal (time point 0) is a very reliable indicator for the necessity of providing the advanced treatment to the diarrhoeic calves. The corresponding value of DSn and DSp for this cut-off point was

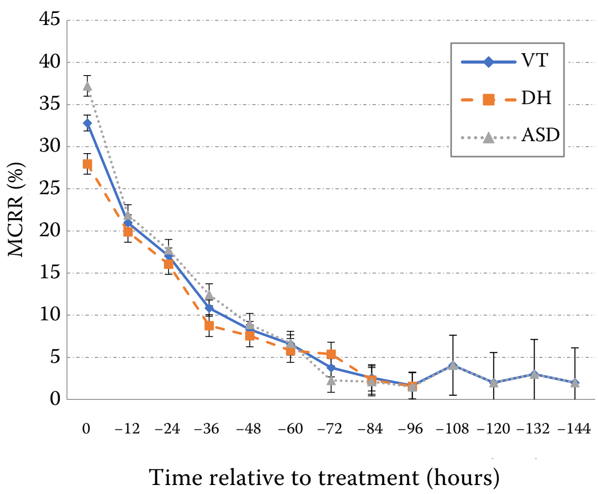


Figure 1. The mean ± SEM milk consumption reduction rate (MCRR %) recorded at different time points relative to the advanced treatment administration in the group of diarrhoeic calves that needed the advanced treatment (VT) and in the subgroups of animals that only had dehydration (DH) or acidosis-septicaemia with or without dehydration (ASD)

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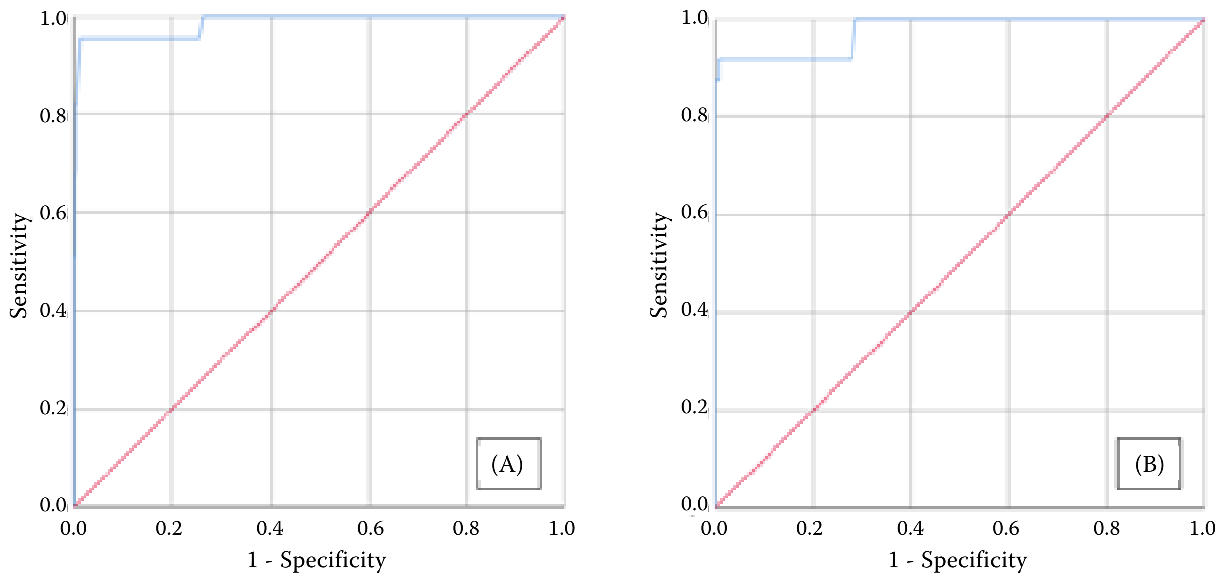


Figure 2. The receiver operating characteristic (ROC) analysis curve for the milk consumption reduction rate at the time point of the advanced treatment administration as an indicator for the detection of diarrhoeic calves that need advanced treatment (A) and those that have acidosis-septicaemia with or without dehydration (B)

very high, at 95.6% and 98.7%, respectively (AUC: 0.987, 95% CI: 0.971–1.000; Figure 2A), suggesting that only a small proportion of calves will be left untreated. The analysis further revealed that using the cut-off point of ≥ 29.6% for the MCRR, the MC on time point 0 is also a very reliable indicator for identifying the diarrhoeic calves with acidosis-septicaemia as the corresponding DSn and DSp was higher than 90% (DSn: 91.7%, DSp: 99.1%; AUC: 0.976, 95% CI: 0.945–1.000; Figure 2B). It should have been expected that this cut-off point would be higher than that which was identified for the necessity of veterinary treatment since acidosis-septicaemia is associated with a considerable decline in MC. The most likely explanation is that the calves were identified early as acidotic-septicaemic and treated before the development of severe clinical signs. Taking into account that the MC declines significantly every 12 h during the last 36 h before the treatment, it was further attempted to evaluate the MCRR at the offered meal at −12 h relative to the treatment administration as a possible indicator for veterinary assistance. The analysis showed that using the cut-off point of ≥ 16.5%, the MC at this time point is a good, but less reliable, indicator than that of time point 0. Despite the low DSn and DSp (DSn: 86.7%, DSp: 90.7%; AUC: 0.958, 95% : 0.940–0.976), this cut-off could be used as an alarm point for the closer monitoring and more frequent standard therapy of the diarrheic calf.

## DISCUSSION

According to previous reports, despite the observed significant changes in the feeding behaviour in limited fed sick calves, the majority of which had diarrhoea, the MC remained practically unaffected during the course of the disease (Svensson and Jensen 2007; Borderas et al. 2009). However, no information regarding the clinical condition of these calves is provided. The results obtained here are similar with those reports as far as it concerns the ST group where the calves did not have any severe clinical signs. In contrast, Sutherland et al. (2018) observed that the MC declined prior to the onset of the symptoms of neonatal diarrhoea syndrome in *ad libitum* fed calves. This deviation from our findings could be partially attributed to the different amount of milk offered to the calves compared to the present study. In support to this point of view, Borderas et al. (2009) observed a significant reduction in the MC on the first days of the disease in *ad libitum* fed calves, whereas no such difference was noted in the limited fed ones. On the other hand, in the study of Sutherland et al. (2018), the calves were defined as having neonatal diarrhoea syndrome when they were presented with diarrhoea and a rectal temperature ≥ 39.5 °C. Such calves were classified in the VT group and especially in the ASD subgroup in the present study; the MCRR in this subgroup on day 0,

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which is the first day that these symptoms were identified, appears to be similar to that observed by Sutherland et al. (2018).

In conclusion, the results of the present study suggest that monitoring milk consumption is a reliable help for the farmer in identifying diarrhoeic calves that need advanced treatment. Hence we suggest that farmers seek veterinary assistance when the consumption of milk by limited fed calves is reduced by 24.5% or more and to implement closer monitoring and provide more intensive care to their calves when the milk intake is reduced by more than 16.5%.

## Conflict of interest

The authors declare no conflict of interest.

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