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Viability of the rapid test of BHB in milk for the diagnosis of bovine ketosis

Viabilidade do teste rápido de BHB no leite para diagnostico de cetose bovina

Viabilidad de la prueba rápida de BHB en leche para el diagnóstico de cetosis bovina

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Abstract
Ketosis in dairy cattle is a metabolic disorder caused by negative energy balance in the postpartum period. It is an attempt to supply the lack of free glucose using the reserve fat to produce ketone bodies (CC), which are acetone, aceto acetate and β-hydroxybutyrate (BHB). Ketosis causes problems due to the accumulation of CC, lack of appetite and consequent loss of body condition score and possible secondary illness due to decreased food intake. Early detection of changes in the health of the dairy cow allows interventions to be taken before the animals' production levels, or even their survival, are at risk. The objective of this work was to evaluate the feasibility of using a rapid test in milk for BHB in postpartum dairy cows in the municipality of Carambeí-PR. Fifty Holstein dairy cows were evaluated in the postpartum period. Blood and milk samples were taken simultaneously. The reagent strip was used in the Health Mate BHB Milk (DFI), and in its control, a rapid test device for BHB in Ketovet blood was used. The tests were repeated at 4, 7 and 14 days postpartum, totaling 150 tests. There was a positive correlation between the rapid test performed with the DFI reagent strip and the blood test. At 7 to 14 days they are the most indicated for the diagnosis of ketosis, using the reagent strip in the milk, and on the 14th day there is the strongest correlation of 78% between the tests, and with a variation of 2% in the indication or not from treatment. The reagent strip has the advantages of performing the diagnosis quickly and at a low cost in the field, making it possible to avoid health and financial losses.
Keywords: Betahydroxybutyrate; Health Mate BHB Milk; Negative energy balance.

Resumo
A cetose em bovinos leiteiros é um distúrbio metabólico causado pelo balanço energético negativo no pós-parto. É uma tentativa de suprir a falta de glicose livre utilizando a gordura de reserva para produção de corpos cetônicos (CC), sendo eles a acetona, o aceto acetato e o β-hidróxibutirato (BHB). A cetose causa problemas pelo acúmulo dos CC, falta de apetite e consequente perda de escore de condição corporal e possível enfermidade secundária pela diminuição na ingestão de alimentos. A detecção precoce de alterações na saúde da vaca leiteira permite que intervenções possam ser tomadas antes que os níveis de produção dos animais, ou até mesmo sua sobrevivência esteja em risco. O objetivo do trabalho foi avaliar a viabilidade do uso de teste rápido no leite para BHB em vacas leiteiras pós-parto no município de Carambeí-PR. Foram avaliadas 50 vacas holandesas de aptidão leiteira em período pós-parto. Foram retiradas amostras de sangue e leite simultaneamente. Foi usada a fita reagente no leite Health Mate BHB Milk (DFI), e na sua contraprova foi usado aparelho de teste rápido para BHB no sangue Ketovet. Foram repetidos os testes com 4, 7 e 14 dias pós-parto totalizando 150 testes. Houve correlação positiva entre o teste rápido realizado com a fita reagente DFI e o teste de sangue. Aos 7 a 14 dia são os mais indicados para o diagnóstico da cetose, utilizando a fita reagente no leite, sendo que no 14 dia se apresenta a correlação mais forte de 78% entre os testes, e com uma variação de 2% na indicação ou não do tratamento. A fita reagente possui as vantagens de realizar o diagnóstico de forma rápida e com baixo custo à campo, possibilitando evitar perdas sanitárias e financeiras.
Palavras-chave: Betahidroxibutirato; Health Mate BHB Milk; Balanço energético negativo.

Resumen
La cetosis en el ganado lechero es un trastorno metabólico causado por un balance energético negativo en el período posparto. Se trata de suplir la carencia de glucosa libre utilizando la grasa de reserva para producir cuerpos cetónicos (CC), que son acetona, acetoacetato y β-hidroxibutirato (BHB). La cetosis causa problemas debido a la acumulación de CC, falta de apetito y la consiguiente pérdida de puntaje de condición corporal y posible enfermedad secundaria debido a la disminución de la ingesta de alimentos. La detección temprana de cambios en la salud de la vaca lechera permite realizar intervenciones antes de que los niveles de producción de los animales, o incluso su supervivencia, estén en riesgo. El objetivo de este trabajo fue evaluar la viabilidad del uso de una prueba rápida en leche para BHB en vacas lecheras en posparto en el municipio de Carambeí-PR. Cincuenta vacas lecheras Holstein fueron evaluadas en el puerperio. Se tomaron muestras de sangre y leche simultáneamente. La tira reactiva se utilizó en el Health Mate BHB Milk (DFI), y en su control se utilizó un dispositivo de prueba rápida para BHB en sangre Ketovet. Las pruebas se repitieron a los 4, 7 y 14 días posparto, totalizando 150 pruebas. Hubo una correlación positiva entre la prueba rápida realizada con la tira reactiva DFI y el análisis de sangre. A los 7 a 14 días son los más indicados para el diagnóstico de cetosis, utilizando la tira reactiva en la leche, y al día 14 se presenta la correlación más fuerte del 78% entre las pruebas, y con una variación del 2% en la indicación o no del tratamiento. La tira reactiva tiene la ventaja de realizar el diagnóstico de forma rápida y económica en el campo, lo que permite evitar pérdidas sanitarias y económicas.
Palabras clave: Betahidroxibutirato; Leche Health Mate BHB; Balance energético negativo.

1. Introduction

World milk production spans many countries around the world. It is considered a commodity, due to the fact that it generates milk 365 days a year (Delamura et al., 2020). In Brazil, Paraná is the state that produces the most milk in Brazil, highlighting the cities of Carambeí and Castro, recognized for the evolution of dairy farming year by year (Júnior; Jung, 2017; Clock et al., 2021).

There is great interest in studying the metabolism of high-yielding dairy cows, especially during the transition period. The transition period consists of two phases, the first being formed by the last three weeks before delivery and the second by the first three postpartum weeks (Grummer, 1995; Alvarenga et al., 2015).

In this transition period, specifically in the postpartum period, ketosis can occur. Ketosis is caused due to negative energy balance (BEN), where insulin levels decrease greatly, the animal's metabolism responds by mobilizing its adipose tissue by increasing non-esterified fatty acids. They are synthesized in the liver producing ketone bodies and beta-hydroxybutyrate (BHB) (McART et al., 2012). The cow's organism undergoes a major change with a 30% decrease in dry matter, decreasing the energy acquired by food intake causing BEN (Faoro & Cardoso, 2019).

Ketosis is a disorder of energy metabolism mainly in high production cows, it is identified clinically and subclinically, it causes loss of milk production, reproductive difficulty and even displacement of abomasum, decrease in milk production, delay of the next calving. and development of secondary diseases (De Freitas et al., 2020). According to McArt et al. (2012), some surveys revealed that at least 17.5% of high production herds show signs of clinical ketosis and that 43.2% have subclinical ketosis.

Clinical ketosis is represented in 4 different ways: primary, secondary, food and spontaneous, in primary the animal does not receive the amount of food needed, in secondary the intake is reduced due to some disease. In the feeding with large intake of foods rich in ketogenic and spontaneous, the animal has high concentrations of ketogenic even with an adequate diet (Van Cleef et al., 2009).

Subclinical ketosis can be diagnosed with a pre-stage of clinical ketosis, where the elevation of ketone bodies occurs without any visualization as in clinical ketosis, this represents a major problem because its silent evolution is diagnosed only with laboratory analysis (Delamura et al., 2020).

For the diagnosis of the disease, tests are performed to estimate BHB in blood, urine and milk (Geishauser et al., 1998; Geron et al., 2018). Diagnosis through blood sampling is performed with a portable electronic device to detect increased

levels of ketone bodies, especially BHB, BHB and blood glucose are measured. An electrochemical reaction occurs between the BHB and the reagent on the instrument strip, generating an electric current. The size of the stream is proportional to the concentrations of BHB in mmol/L of blood (Iwersen, 2009). However, it is a test that requires the removal of the animal's blood and the rural producer must have the device.

Urinalysis is another way of diagnosing ketosis. The collection is performed through gentle massage in the perineal and vulvar region. The analysis of these substances must be carried out within a maximum of 30 minutes after collection, because after this period, the reduction in concentrations can reach up to 40% (Van Cleef, 2009).

Studies have also been carried out to determine BHB in milk samples, due to the ease of obtaining samples and the fact that BHB is stable, unlike other ketone bodies, which are volatile (Tharwat et al., 2012). With this objective, a semiquantitative method has been developed, based on dry chemistry (use of reagent strips), which produces a violet color reaction, whose intensity is proportional to the concentration of BHB in the sample. Rapid tests can be accurate and present a good cost benefit to the producer, in addition to speed and agility.

However, there is some suspicion by the rural producer of the efficiency and accuracy of these rapid tests in milk using reagent strip in relation to BHB blood. Within this context, the present study aimed to evaluate the feasibility of using the rapid milk test for BHB in postpartum dairy cows, comparing the accuracy of the two methods (milk and blood). In addition to checking whether it is recommended to replace the blood test with the rapid test strip in milk.

2. Methodology

The work was carried out in the municipality of Carambeí, state of Paraná, on a private property, which performs two to three mechanized milkings per day. The rearing systems used are semi-extensive, intensive and compost barns, with a pre-established diet by nutritionists specializing in dairy cattle.

Fifty dairy cows (Holstein Hpb and Hvb) in the postpartum phase were evaluated. Cows were evaluated at 4, 7 and 14 days postpartum for the prevalence and degree of clinical and subclinical ketosis, using a BHB measurer in milk and blood. The animals were not subjected to tests, only the material collected.

For the milk tests, milk was collected after 4, 7 and 14 days postpartum from each animal. A composite sample of the four teats was performed after pre-dipping using gloves and a sterile Falcon tube. Immediately after collection, a single immersion of the tape in the milk was performed for a maximum of 2 seconds, removing the excess milk from the edge of the container, without harming the reading area, and then 60 seconds were timed using the UnidoctorVet device, this and the exact time for reading the test, which can also be manually timed, after this period, a reaction occurs on the strip causing a color in some cases cream to red, depending a lot on the concentration of BHB in the milk (Figure 1).

Figure 1 – Tests performed on milk during the experiment, Carambeí, Paraná, 2021.

Source: Authors.

The milk test was carried out within a maximum of 1 hour after collection. When the immediate test was not possible, the milk sample was kept cold for up to 12 hours, in which case the milk must reach room temperature before the test (25 to 30°C), for the test to be carried out. The tape container was always kept closed, avoiding contact with moisture as much as possible.

Along with the milk test, the blood test was performed (Figure 2). In this test, a small percentage of blood was removed from the lower end of the tail (tail) of the animal by puncturing the coccygeal arteriovenous complex with a 40x12mm needle and adding a drop of blood (0.7µL) to a reagent strip. for BHB, and later inserted in the portable equipment Ketovet of Eco diagnostica. The device indicates levels, ranging from 0.0mmol/L to 5.0mmol/L.

Figure 2 – Conducting blood tests, Carambeí, Paraná, 2021.

Source: Authors.

For the quantification of the result in the comparison of both tests, notes were defined; because the ribbon test used a polychromatic scale and the blood numerical scale, it was possible to compare the color of the ribbon with the blood value. Thus, a scale of grades of 1 and 2 was stipulated, where 1 is indicated to carry out the treatment and 2 not to carry out the treatment, based on the polychromatic scale present on the packaging where its color varies from 0 to 1000, a correlation with blood values and their respective grade (Table 1).

Table 1 – Correlation of scores between blood and milk, Carambeí, Paraná, 2021.

NOTA	2		1		
BHB LEITE	0	100	200	500	1000
"					
BHB SANGUE	0,0mmol/L - 1,1mmol/L		1,2mmol/L - 5,0mmol/L		

Source: Authors.

Thus, an evaluation was carried out by 3 evaluators (zootechnician, veterinarian and the author) who provided the respective notes for each evaluation performed on the BHB in milk (Figure 3).

Figure 3 - Example of evaluations, carried out on the reagent strip by different evaluators to award grades, Carambeí, Paraná, 2021.

Source: Authors.

After the tests to measure BHB in milk and blood, the data were tabulated and submitted to correlation analysis performed with the aid of Microsoft Excel 2007® and the results interpreted. A comparison of the accuracy of the two proposed methods was performed, comparing whether the levels of BHB are the same in both tests.

3. Results and Discussion

The test performed by examining the milk with a reagent strip to indicate positivity or negativity of ketones obtained results of 42.0% (21/50), 36.0% (18/50) and 18.0% (9/50) of animals positive for ketosis on the fourth, seventh and fourteenth days postpartum to indicate treatment or not assigned their respective scores 1 (Treat) and 2 (Do Not Treat). As well as the tests carried out in the blood showed 34.0% (17/50), 38% (19/50) and 16% (8/50) on the fourth, seventh and fourteenth days of animals that showed concentration indicative of subclinical ketosis. ≥ 1.2 mmol/L (Table 2).

Table 2 - Values measured by means of a reagent strip test in the milk and their respective scores 1 (Treat) and 2 (Do not Treat) and the blood values by a portable device evaluating the plasma levels of beta-hydroxybutyrate (BHB) in cows of the breed Dutch. Carambeí, Paraná, 2021.

Animal	Milk (µmol/l)						Blood (mmol/l)		
	Day 4	TREAT	Day 7	TREAT	Day 14	TREAT	Day 4	Day 7	Day 14
1	200	1	500	1	100	1	0,9	1	1
2	100	2	100	2	100	2	0,8	1	1
3	100	2	100	2	100	2	0,9	1	0,8
4	100	1	100	1	0	2	1,5	1,5	1,1
5	200	1	200	1	100	2	1,6	1,1	1
6	200	2	100	1	100	2	1,4	1	1,1
7	100	2	100	2	100	2	1	1	1,5
8	100	1	100	1	100	2	1,4	1,1	0,8
9	100	1	100	2	100	2	0,7	0,6	1
10	100	1	100	1	100	2	0,8	1,2	0,8
11	100	2	100	2	200	1	0,7	0,8	0,7
12	100	1	500	1	100	2	1,9	2,2	0,6
13	200	2	100	2	100	2	0,9	1,2	1
14	200	1	100	1	100	1	2,2	1,3	1
15	200	1	100	2	100	2	2,4	1,1	0,5
16	100	1	0	2	100	2	1,6	1,2	1
17	100	1	0	2	100	2	1,3	1,3	0,7
18	0	2	200	1	100	2	0,9	1,4	1,1
19	100	2	100	2	100	2	1	0,9	0,9
20	100	2	100	2	100	2	1,2	0,9	1,1
21	100	1	100	1	100	2	1,1	0,9	0,2
22	100	2	100	1	0	2	1	1,5	0,9
23	100	1	200	1	100	1	1,5	1,5	1,2
24	100	1	100	2	100	2	0,9	1,1	1
25	100	2	100	2	100	2	0,6	1,4	1
26	100	1	100	2	100	2	1	1,1	1
27	100	2	100	2	100	2	1	0,8	1,1
28	100	2	100	2	200	1	0,9	1,1	1,4
29	100	2	100	2	0	2	1,1	1,2	0,8
30	0	2	100	2	100	2	2,1	1,5	0,9
31	100	1	100	2	100	2	1,9	0,6	0,1
32	200	1	200	1	200	1	1,6	1,8	1,8
33	100	1	500	1	100	2	1,5	1,5	0,6
34	100	2	100	2	100	2	1	0,6	0,6
35	100	2	100	2	100	2	0,6	0,9	1,1
36	100	2	100	2	100	2	0,6	1,1	1
37	100	2	100	2	100	2	0,6	1	0,8
38	100	1	100	2	100	2	0,8	1,1	0,9
39	100	2	100	2	100	2	1	1	0,9
40	100	2	100	2	0	2	0,8	0,8	0,3
41	100	1	100	2	100	2	0,9	0,8	1,7
42	100	2	100	1	100	2	1,3	1,1	1,2
43	100	1	100	2	100	1	0,7	1,1	1,1
44	100	2	200	1	100	2	1	1,5	0,8
45	100	2	100	2	100	2	0,8	1,5	1,2
46	100	2	100	2	100	2	1	1	0,7
47	100	2	100	2	100	2	0,5	1	1
48	100	2	200	1	0	2	0,9	1,3	0,7
49	100	2	100	2	100	1	1,5	0,7	1,3
50	0	2	200	1	100	1	0,7	1,4	1

Source: Authors.

In the general average obtained in the present experiment, the concentrations of BHB are within the recommended by Smith et al. (2006), that is, less than 1.2 mmol/L of BHB per sample. However, there are some animals, such as the fifteenth that presented higher values in the blood test (2.4 mmol/L of BHB) (Table 2).

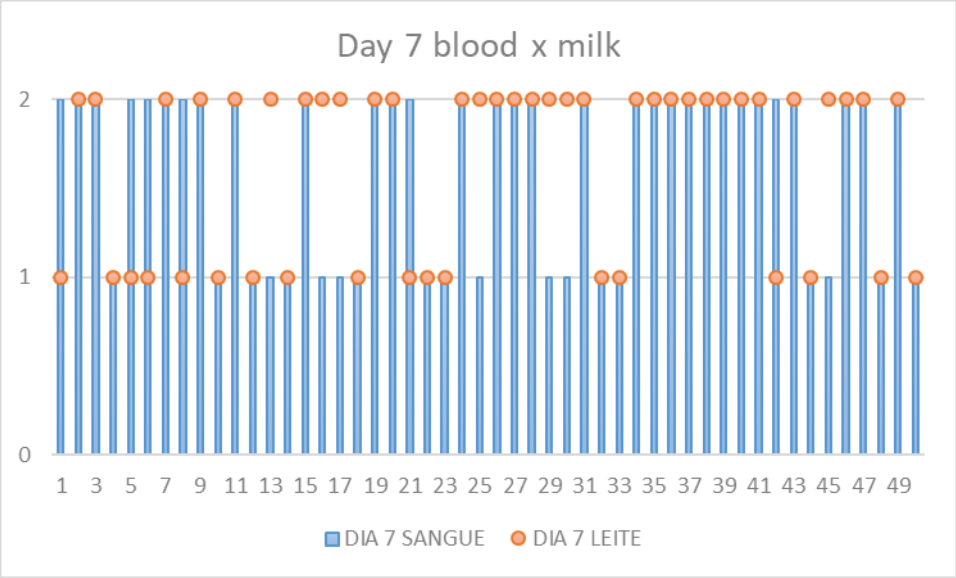
Cows with BHB between 1.2 to 2.9 mmol/L are references for diagnosing subclinical ketosis, BHB values greater than 3 mmol/L are considered clinical ketosis, the increase in ketone bodies is accompanied by hypoglycemia (OETZEL, 2004).

The evaluations carried out on the 4th postpartum day showed a correlation of 72% between both tests, where in 24% of the time both tests indicated treatment and 48% indicated not performing the treatment, with this it can be deduced that on the 4th day postpartum BHB levels are not decisive for the indication of treatment or not.

Evaluating both tests individually on the 4th day, it was observed that the blood test indicated the treatment in 34% of the time while the milk test 42%, with this there is a percentage variation of 8% between both tests.

The evaluations that were performed on the 7th postpartum day showed a correlation of 74% between both tests (Graph 1) where in 24% of the time both tests indicated treatment and 50% indicated not performing treatment, based on these results. their similarity was observed between the 4th and 7th days.

Graph 1 – Relationship between both tests on the 7th day from the pre-established grade. Carambeí, Paraná, 2021.

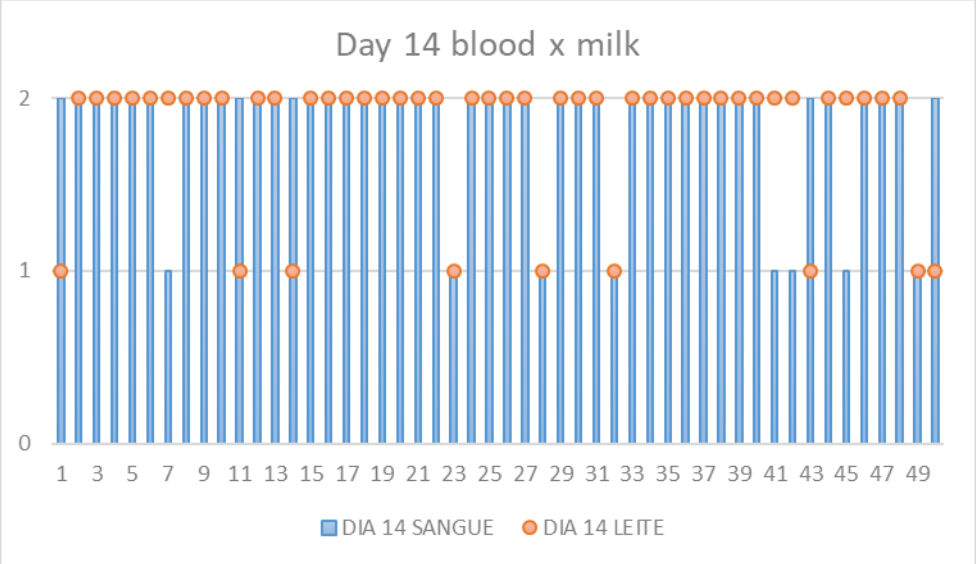


Source: Authors.

Performing the individual evaluation between the tests on the 7th day, it was possible to observe that the blood test indicated the treatment in 38% of the time while the milk test 36% (Graph 1), indicating a percentage variation of 2% between both tests, this indicates a good relationship between the milk and blood test being a suitable day for the diagnosis of ketosis.

On the last postpartum evaluation day, that is, on the 14th day, the best correlation between both tests was observed, in 78% of the tests the results were compatible, thus indicating the greatest similarity between the tests on the 14th day (Graph 2).

Graph 2 - Relationship between both tests on the 14th day from the pre-established grade. Carambeí, Paraná, 2021.



Source: Authors.

The evaluations carried out individually between the blood and milk test, showed the treatment in the blood in 16% of the time and 18% in the milk. Thus, based on the results, it is possible to diagnose ketosis and its need for treatment or not, between 7 and 14 days using the reagent strip in the Health Mate BHB Milk (DFI). It is possible to carry out the diagnosis quickly and at low cost in the field, making it possible to avoid health and financial losses to the herds.

For each increase in BHB in the concentration of 0.1 mmol/L in the blood, there is an increase in the risk of displacement of the abomasum and considerable disposal of animals that die, in addition to a drop in the production of 0.5 kg of milk in the first 30 days of lactation (Mc Art et al., 2012; Schneider et al. 2020).

Another relevant factor in the control of postpartum diseases, such as ketosis, is the control of the score in dairy cows. The recommended value for disease prevention is 3.5 to 4.0 (from Carvalho et al., 2019). Lago et al. (2001) suggested that calving cows without adequate body reserve may be more prone to infectious diseases, metabolic disorders, low reproductive efficiency and reduced milk production, while excessively fat cows would be more predisposed to calving difficulties, fat cow syndrome. and sometimes death.

Edmonson et al. (1989) found that cows with high body condition (4 - 4.25) are more prone to ketosis, due to the fact that they have lower consumption soon after calving and mobilize more body reserves.

Technological advances are often evolving, the producer needs to adapt the conditions to maintain a good level of productivity and quality, investing in his property (Clock et al., 2021; Gabardo et al., 2021; Bauchrowitz et al., 2022; Gabardo et al., 2022). The diagnosis of herd health is of great importance for the control of diseases that affect cattle herds, such as ketosis, which causes losses in milk productivity, decreased milk quality and gastrointestinal complications.

The use of tests such as BHB blood and milk using reagent strips make a prior diagnosis of the degree of the disease and its need for treatment. However, the blood test has the disadvantage of collecting blood from animals and owning the device. Comparing the rapid test through milk can be more viable and easier to handle by the producer, due to optimizing production needs. Thinking about practicality and costs involved in diagnoses.

The milk production chain drives the local economy, is a constant source of income for thousands of small producers, generates taxes, keeps people in the field and is a source of jobs. Therefore, it is of paramount importance to carry out the diagnosis of the dairy herd.

4. Conclusion

There was a positive correlation between the rapid test performed with the Health Mate BHB Milk (DFI) reagent strip and the blood test.

The 7th to 14th day are the most indicated for the diagnosis of ketosis, using the reagent strip in the Health Mate BHB Milk (DFI), and on the 14th day there is the strongest correlation of 78% between the tests, and with a variation of 2% in the indication or not of the treatment.

The rapid test of the reagent strip in the Health Mate BHB Milk (DFI) presents similarity with the test in the blood, being possible to use it as a tool for the diagnosis of ketosis and indication of treatment or not.

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