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Note

The influence of milk κ-casein and β-lactoglobulin phenotypes on fatty acid composition of milk from Reggiana cows

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Abstract – The aim of the present study was to examine the association between milk protein polymorphism and fatty acid profiles of bovine milk. Milk samples were collected from each of 55 Reggiana cows during early, mid- and late lactation, respectively, in two farms within the production area of Parmigiano Reggiano cheese. Identification and quantification of fatty acids were performed by gas chromatography. Milk fatty acid composition using cows of differing κ-casein (κ-Cn) and β-lactoglobulin (β-Lg) phenotypes was investigated. Statistically significant results regarding the associations between milk fatty acid composition and κ-Cn phenotype were found; in particular, κ-Cn BB seems to influence de novo fatty acid synthesis in the mammary gland. Also, κ-Cn AB seems to have the same effect. Proportions of $C_{10:0}$ (2.29^{a} AA; 2.53^{b} AB; 2.59^{b} BB), $C_{12:0}$ (2.77^{a} AA; 3.17^{b} AB; 3.20^{b} BB) and $C_{14:0}$ (9.22^{a} AA; 10.25^{b} AB; 10.27^{b} BB) were higher in the milk from cows with κ-Cn phenotypes AB and BB vs. κ-Cn phenotype AA ($P < 0.05$). Conversely, $C_{18:0}$ (7.84^{b} AA; $7.20^{a,b}$AB; 6.94^{a} BB) and $C_{18:1}$ (19.19^{b} AA; 16.81^{a} AB; 16.79^{a} BB) were lower in the milk from cows with κ-Cn phenotypes AB and BB vs. κ-Cn phenotype AA ($P < 0.05$). The association between milk fatty acid composition and β-Lg phenotype was not statistically significant, except for some fatty acids. In particular, $C_{12:0}$ (3.05^{a} AA; 3.04^{a} AB; 3.33^{b} BB) was higher in the milk from cows with β-Lg phenotype BB vs. β-Lg phenotypes AA and AB ($P < 0.05$). Concentrations of $C_{18:0}$ (6.93^{a} AA; 7.86^{b} AB; 6.59^{a} BB) and $C_{18:1}$ ($16.74^{a,b}$AA; 18.24^{b} AB; 16.07^{a} BB) were lower in the milk from cows with β-Lg phenotypes AA and BB vs. β-Lg phenotype AB ($P < 0.05$).

fatty acids / genetic polymorphism / κ-casein / β-lactoglobulin / protein

摘要 – 牛乳 κ-酪蛋白与 β-乳球蛋白表型对 Reggiana 母牛牛乳中脂肪酸组成的影响。本研究目的是检测牛乳蛋白多态性和牛乳中脂肪酸组成之间的相关性。牛乳样品来自 Parmigiano Reggiano 干酪产区的两个农场的各 55 头 Reggiana 母牛, 分别在哺乳期的早、中、晚三个时期取样。采用气相色谱对牛乳中脂肪酸进行鉴定和量化。牛乳中脂肪酸的组成与奶牛的 κ-酪蛋白和 β-乳球蛋白的表型的相关性被调查。牛乳脂肪酸的组成和 κ-酪蛋白表型显著相关, 尤其 κ-酪蛋白 BB 表型影响奶牛乳腺中脂肪酸的从头合成。 κ-酪蛋白 AB 表型也有相同的影响。带有 κ-酪蛋白 AB 、BB表型 与 κ-酪蛋白 表型 AA 的奶牛相比, 所产的牛奶中 $C_{10:0}$ (2.29^{a} AA; 2.53^{b} AB; 2.59^{b} BB), $C_{12:0}$ (2.77^{a} AA; 3.17^{b} AB; 3.20^{b} BB) 和 $C_{14:0}$ (9.22^{a} AA; 10.25^{b} AB; 10.27^{b} BB) 型脂肪酸的含量较高, 而 $C_{18:0}$ (7.84^{b} AA; $7.20^{a,b}$AB; 6.94^{a} BB) 和 $C_{18:1}$ (19.19^{b} AA; 16.81^{a} AB; 16.79^{a} BB) 脂肪酸的含量较低。除了部分脂肪酸外, 牛乳脂肪酸组成和 β-乳球蛋白表型之间相关性不显著。尤其, β-乳球蛋白 BB 表型 与 β-乳球蛋白 AA、AB

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表型的奶牛相比, 产出的牛乳中 $C_{12:0}$ (3.05[a] AA; 3.04[a] AB; 3.33[b] BB) 脂肪酸含量较高, 而 β-乳球蛋白 AA 、 BB 表型与 β-乳球蛋白 AB 表型相比, $C_{18:0}$ (6.93[a] AA; 7.86[b] AB; 6.59[a] BB) 和 $C_{18:1}$ (16.74[a,b]AA; 18.24[b] AB; 16.07[a] BB) 脂肪酸的含量较低。

脂肪酸 / 遗传多态性 / κ-酪蛋白 / β-乳球蛋白 / 蛋白质

Résumé – Influence du phénotype de la caséine κ et de la β-lactoglobuline sur la composition en acides gras du lait de vaches Reggiana. Le but de l'étude était d'examiner l'association entre le polymorphisme des protéines laitières et les profils d'acides gras du lait de vache. Des échantillons de laits individuels de 55 vaches de race Reggiana ont été collectés en début, milieu et fin de lactation respectivement, dans deux fermes de l'aire de production du fromage Parmigiano Reggiano. L'identification et la quantification des acides gras ont été réalisées par chromatographie en phase gazeuse. La composition en acides gras du lait de vaches de différents phénotypes de la caséine κ et de la β-lactoglobuline a été étudiée. Des résultats statistiquement significatifs ($P < 0,05$) concernant les associations entre composition en acides gras du lait et phénotype de caséine κ ont été trouvés, la caséine κ AB semblant influencer la synthèse de novo d'acides gras dans la glande mammaire. La caséine κ AB semblait aussi avoir le même effet. Les proportions de $C_{10:0}$ (2,29[a] AA ; 2,53[b] AB ; 2,59[b] BB), $C_{12:0}$ (2,77[a] AA ; 3,17[b] AB ; 3,20[b] BB) et $C_{14:0}$ (9,22[a] AA ; 10,25[b] AB ; 10,27[b] BB) étaient plus élevées dans le lait des vaches ayant les phénotypes de caséine κ AB et BB que le phénotype AB. À l'inverse, les proportions de $C_{18:0}$ (7,84[b] AA ; 7,20[a,b] AB ; 6,94[a] BB) et $C_{18:1}$ (19,19[b] AA ; 16,81[a] AB ; 16,79[a] BB) étaient plus faibles dans le lait des vaches ayant les phénotypes de caséine κ AB et BB par rapport au phénotype AA. L'association entre composition du lait en acides gras et phénotype de la β-lactoglobuline n'était pas statistiquement significative excepté pour quelques acides gras, en particulier le $C_{12:0}$ (3,05[a] AA ; 3,04[a] AB ; 3,33[b] BB) était plus élevé dans le lait de vaches ayant le phénotype de la β-lactoglobuline BB par rapport aux phénotypes AA et AB. Les concentrations en $C_{18:0}$ (6,93[a] AA ; 7,86[b] AB ; 6,59[a] BB) et $C_{18:1}$ (16,74[a,b] AA ; 18,24[b] AB ; 16,07[a] BB) étaient plus faibles dans le lait des vaches ayant les phénotypes de la β-lactoglobuline AA et BB vs. le phénotype AB.

acides gras / polymorphisme génétique / caséine κ / β-lactoglobuline / protéine

1. INTRODUCTION

Cow's milk contains from 3 to 5% fat (that is subject to individual and seasonal variations), which is present in the form of spherical globules synthesized by the secretory cells of the mammary gland epithelium [25]. The size of the fat globules varies from 0.1 μm to 20 μm, with an average of about 3–4 μm, and may be influenced by the breed, the lactation stage, diet and the season. They are surrounded by a membrane of about 10–20 nm thickness, that has a rather complex composition that varies, due to several factors (diet, breed, health and lactation stage). It consists mainly of phospholipids, glycolipids, proteins, lipoproteins and enzymes including butyrophilin, xanthine oxidase and adipophilin [9]. The membrane also plays a fundamental role in the stability of the fat globules.

The fatty acids present in milk fat originate from different sources [14] and they are affected by various factors, both endogenous (breed, individual milk-production, state of health, lactation stage) as well as exogenous (environmental conditions and farm management, with special reference to the type of diet [23]).

In the last few years, there has been an increase in studies concerning the influence of the fatty acid composition and the other components of milk on the technological and sensory properties [18, 19]. The connection between the fatty acid composition and the different genetic polymorphism of proteins has also been studied [3, 5]. In a study by Bobe et al. [4],

an association was taken into consideration between the different κ-casein (κ-Cn) and β-lactoglobulin (β-Lg) phenotypes and the fatty acid composition of milk, with special reference to certain fatty acids synthesized in the mammary gland.

Among the 11 genetic variants of κ-Cn, A and B are the most frequent [10]. Milk with κ-Cn B is more appropriate than milk with κ-Cn A for dairy product manufacture because it contains smaller casein micelles which coagulate faster and form a more uniform curd, resulting in a higher cheese yield [21]. These features attributed to variant B have also been confirmed by more recent studies [12, 24].

As regards whey proteins, the most important protein is β-Lg. Ever since Aschaffenburg and Drewry [1] encountered its polymorphism for the first time, 11 genetic variants of this protein have been identified to date [13, 20] and variant A and variant B are the most frequent variants.

On the basis of these considerations, particularly by evaluating the fatty acid composition in relation to different factors, the purpose of this study was to confirm the results obtained by Bobe et al. [4]. So the possible existence of an interaction between protein phenotype and milk fatty acid composition was investigated using Reggiana cows of differing κ-Cn and β-Lg phenotypes.

2. MATERIALS AND METHODS

2.1. Selection of animals and collection of milk samples from farms

This study was conducted in two farms with Reggiana dairy cows within the production area of Parmigiano Reggiano cheese located in the province of Reggio Emilia. Cows were fed with the basic food ration according to the disciplinary regulations of the Parmigiano Reggiano Consortium. In particular, cows were fed with preserved forage (hay) and integrated composite feed (1 kg·3 L^{-1} milk) in the period December–March, while they were fed with fresh forage and integrated composite feed (1 kg·3 L^{-1} milk) in the period April–November.

Table I. Distribution of κ-casein and β-lactoglobulin phenotypes among Reggiana cows.

κ-Casein phenotype	β-Lactoglobulin phenotype			
	AA	AB	BB	Total
AA	3[a]	5		8
AB	10	9	8	27
BB	6	8	6	20
Total	19	22	14	55

[a] Number of cows.

In the two farms, 55 Reggiana dairy cows were selected between their 2nd and 5th calving for the experiment. The Reggiana cows had different κ-Cn and β-Lg phenotypes (Tab. I).

A total of 165 samples, milk samples from each cow, were collected at three different points of the lactation stage (early lactation = approximately 60 d after calving; mid-lactation = approximately 120 d after calving; late lactation = approximately 180 d after calving). Therefore, the effects of the lactation curve, the seasonal variations and the different diet rations, which can influence the composition of the milk and the physico-chemical properties of the fat, were eliminated.

2.2. Characterization of milk protein genetic polymorphism

Information about genetic polymorphism of milk proteins, particularly of κ-Cn and β-Lg, was obtained from the Provincial Breeders' Association. Characterization was carried out by means of electrophoresis [8].

2.3. Sample treatment

The individual milk samples were taken in duplicate in 150-mL containers during the evening's milking and were kept at 2–4 °C for 24 h.

2.4. Determination of the gross composition of milk samples

Milk samples were analyzed to determine casein and fat content by means of a Milkoscan FT-IR120 (Foss Analytical A/S, Hilleroed, Denmark). The determination of pH was carried out using the Orion pH meter at a temperature of 20 °C. Titratable acidity was determined by titration of 50 mL of milk with 0.25 mol·L^{-1} NaOH in the presence of phenolphthalein and was expressed in °SH (Soxhlet-Henkel). Somatic cell counts were determined using the fluoropto-electronic method by means of a Fossomatic [22].

2.5. Determination of the fatty acid composition of milk samples

Extraction of milk fat from 165 individual milk samples was carried out in duplicate, using the Röse-Gottlieb method [2].

The fatty acid profile of milk samples was determined after fatty acid transesterification into fatty acid methyl ester (FAME) using KOH in 2 mol·L^{-1} methanol, as described by Christie [6].

The analysis of fatty acid methyl esters (FAME) was carried out using a Perkin Elmer Clarus 500 model gas chromatograph equipped with a Restek 2330 capillary column (30 m × 0.25 mm i.d. × 0.2 μm, cyanopropylic), a flame ionization detector (FID) and an autosampler. The FID detector was maintained at 250 °C with an air flow of 400 mL·min^{-1} and a hydrogen flow of 40 mL·min^{-1}. The injector was maintained at 250 °C with a 1:20 split ratio. A volume of 1 μL was injected. The temperature of the column was programed as follows: from 40 °C to 160 °C within 12 °C·min^{-1}; from 160 °C to 200 °C within 10 °C·min^{-1} and from 200 °C to 240 °C by 10 °C·min^{-1}. The total time of the chromatography run was 23 min.

Methyl-pelargonate (Sigma-Aldrich, Milan, Italy) was used as an internal standard (C_9 methyl-pelargonate, 1 mg·mL^{-1}).

2.6. Statistical analysis

Analysis of the multivariate variance, using the general linear models (GLM) procedure was carried out using the SPSS package for Windows, version 13.0 (SPSS Inc., Chicago, Illinois, USA). Tukey's HSD test was used to obtain comparisons among sample means at the 5% significance level ($P < 0.05$).

3. RESULTS AND DISCUSSION

Tables II and III show the gross composition and somatic cell count of milk samples grouped according to the genetic polymorphism of κ-Cn and β-Lg.

Evaluation of the results did not show significant differences among the means and they were in accordance with the literature reports.

Tables IV and V show the composition of fatty acids of the milk samples with different κ-Cn and β-Lg phenotypes. In this study eleven major fatty acids were identified in bovine milk, confirming the results of Bobe et al. [4]. The sum of fatty acids from $C_{6:0}$ to $C_{14:0}$ was performed as an indicator of de novo synthesis in the mammary gland [11]. The genetic polymorphism of κ-Cn and β-Lg had a significant effect; in particular, milk fatty acid composition was influenced by κ-Cn phenotypes with a statistically significantly higher concentration ($P < 0.05$) of capric acid ($C_{10:0}$),

Table II. Physico-chemical characteristics and somatic cell count of Reggiana cow's milk with different κ-Cn phenotypes (mean values ± s.d., n = 165 samples).

κ-Cn phenotype	AA	AB	BB
Milk ($kg \cdot d^{-1}$)	23.16 ± 5.88	22.76 ± 4.77	23.28 ± 5.46
pH	6.71 ± 0.03	6.72 ± 0.01	6.71 ± 0.02
Titratable acidity ($°SH \cdot 50\ mL^{-1}$)	3.19 ± 0.09	3.34 ± 0.03	3.35 ± 0.04
Casein ($g \cdot 100\ g^{-1}$)	2.60 ± 0.32	2.67 ± 0.21	2.68 ± 0.28
Fat ($g \cdot 100\ g^{-1}$)	3.81 ± 0.12	3.66 ± 0.15	3.82 ± 0.13
Somatic cell count ($10^3 \cdot mL^{-1}$)	155.97 ± 27.55	123.85 ± 16.86	135.83 ± 29.90

AA = 8 cows; AB = 27 cows; BB = 20 cows.

Table III. Physico-chemical characteristics and somatic cell count of Reggiana cow's milk with different β-Lg phenotypes (mean values ± s.d., n = 165 samples).

β-Lg phenotype	AA	AB	BB
Milk ($kg \cdot d^{-1}$)	21.26 ± 5.96	23.64 ± 4.56	23.12 ± 5.80
pH	6.71 ± 0.03	6.72 ± 0.01	6.69 ± 0.01
Titratable acidity ($°SH \cdot 50\ mL^{-1}$)	3.37 ± 0.04	3.28 ± 0.06	3.43 ± 0.06
Casein ($g \cdot 100\ g^{-1}$)	2.70 ± 0.28	2.65 ± 0.26	2.62 ± 0.21
Fat ($g \cdot 100\ g^{-1}$)	3.90 ± 0.09	3.74 ± 0.19	3.53 ± 0.22
Somatic cell count ($10^3 \cdot mL^{-1}$)	159.23 ± 59.09	109.34 ± 19.19	147.51 ± 40.43

AA = 19 cows; AB = 22 cows; BB = 14 cows.

Table IV. Fatty acid composition of Reggiana cow's milk with different κ-Cn phenotypes (mean values ± s.d., n = 165 samples).

Fatty acid composition	κ-Cn phenotype		
(weight % of total fatty acids)	AA	AB	BB
$C_{4:0}$	2.02 ± 0.41	2.04 ± 0.5	1.99 ± 0.47
$C_{6:0}$	1.39 ± 0.21	1.42 ± 0.21	1.42 ± 0.21
$C_{8:0}$	0.96 ± 0.2	1.00 ± 0.18	1.02 ± 0.17
$C_{10:0}$	2.29^{a} ± 0.61	2.53^{b} ± 0.67	2.59^{b} ± 0.55
$C_{12:0}$	2.77^{a} ± 0.73	3.17^{b} ± 1.05	3.20^{b} ± 0.72
$C_{14:0}$	9.22^{a} ± 1.87	10.25^{b} ± 2.44	10.27^{b} ± 2.01
$C_{16:0}$	21.16 ± 3.76	22.76 ± 6.57	22.15 ± 4.58
$C_{16:1}$	1.62 ± 0.47	1.55 ± 0.47	1.59 ± 0.42
$C_{18:0}$	7.84^{b} ± 2.57	$7.20^{a,b}$ ± 2.48	6.94^{a} ± 2.02
$C_{18:1}$	19.19^{b} ± 7.31	16.81^{a} ± 4.79	16.79^{a} ± 4.29
$C_{18:2}$	2.49 ± 0.77	2.60 ± 0.70	2.49 ± 0.68
Sum $C_{6:0}$–$C_{14:0}$	16.62^{a} ± 3.37	18.37^{b} ± 3.78	18.49^{b} ± 3.78

[ab] Means within a row with different superscripts differ at $P < 0.05$.
AA = 8 cows; AB = 27 cows; BB = 20 cows.

Table V. Fatty acid composition of Reggiana cow's milk with different β-Lg phenotypes (mean values ± s.d., n = 165 samples).

Fatty acid composition (weight % of total fatty acids)	β-Lg phenotype		
	AA	AB	BB
$C_{4:0}$	1.97 ± 0.43	2.05 ± 0.52	2.06 ± 0.48
$C_{6:0}$	1.39 ± 0.21	1.42 ± 0.21	1.42 ± 0.2
$C_{8:0}$	0.99 ± 0.18	0.99 ± 0.18	1.03 ± 0.18
$C_{10:0}$	2.47 ± 0.59	2.47 ± 0.58	2.65 ± 0.71
$C_{12:0}$	3.05^{a} ± 0.82	3.04^{a} ± 0.75	3.33^{b} ± 1.20
$C_{14:0}$	9.85 ± 2.27	10.18 ± 2.10	10.35 ± 2.43
$C_{16:0}$	21.72 ± 5.06	22.90 ± 4.81	22.28 ± 7.31
$C_{16:1}$	1.59 ± 0.44	1.56 ± 0.39	1.59 ± 0.55
$C_{18:0}$	6.93^{a} ± 1.81	7.86^{b} ± 2.81	6.59^{a} ± 2.04
$C_{18:1}$	$16.74^{a,b}$ ± 4.27	18.24^{b} ± 5.95	16.07^{a} ± 4.65
$C_{18:2}$	2.50 ± 0.67	2.65 ± 0.81	2.46 ± 0.56
Sum $C_{6:0}$–$C_{14:0}$	17.76 ± 3.62	18.10 ± 3.76	18.78 ± 3.80

[ab] Means within a row with different superscripts differ at $P < 0.05$.
AA = 19 cows; AB = 22 cows; BB = 14 cows.

lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$), and at the same time, lower concentration ($P < 0.05$) of stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$) in milk fat of cows with the BB variant than in milk fat of cows with the AA variant (Tab. IV). Higher proportions of $C_{8:0}$ to $C_{14:0}$ of cows with κ-Cn AB and BB vs. κ-Cn AA were significant at $P < 0.05$ at 60 and 180 days but not at 120 days post-calving. The decrease in proportions of $C_{18:0}$ and $C_{18:1}$ were significant at $P < 0.05$ only at 60 days post-calving. Therefore, the higher proportions of lauric and myristic acid can lead us to suppose that κ-Cn BB can be associated positively with increased de novo fatty acid synthesis in the mammary gland, in accordance with Bobe et al. [4]. Also, κ-Cn AB seems to have the same effect. The sum of fatty acids from $C_{6:0}$ to $C_{14:0}$ ($P < 0.05$) in milk fat of cows with the BB variant is statistically significantly higher than in milk fat of cows with the AA variant.

Milk fatty acid composition was also influenced by β-Lg phenotypes. Samples with β-Lg BB had a statistically significantly ($P < 0.05$) higher concentration of lauric acid ($C_{12:0}$). Concentrations of myristic acid ($C_{14:0}$) and palmitic acid ($C_{16:0}$) were higher in BB samples compared with AA samples but the difference was not statistically significant ($P < 0.05$). Concentrations of stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$) were lower ($P < 0.05$) in milk fat of cows with the AA and BB variants than in milk fat of cows with the AB variant (Tab. V). Higher proportions of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ of cows with β-Lg AB vs. β-Lg AA and BB were significant at $P < 0.05$ only at 60 days post-calving. The higher proportions of $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ of cows with β-Lg BB vs. β-Lg AA were significant at $P < 0.05$ only at 120 days post-calving. The higher proportions of lauric acid, myristic acid and palmitic acid can lead us to suppose that β-Lg BB can be associated positively with increased de novo fatty acid synthesis in the mammary gland, in accordance with Bobe et al. [4]. Also in this case, the sum

of fatty acids from $C_{6:0}$ to $C_{14:0}$ in milk fat of cows with the BB variant is higher than in milk fat of cows with the AA variant but not statistically significant ($P < 0.05$).

These data, therefore, confirm the results obtained by Bobe et al. [4], in which the same fatty acids were taken into account. Specifically, our data confirm those obtained by Bobe et al. as regards the trend of fatty acids in relation to the genetic polymorphism of κ-Cn. However, the profiles of fatty acids in relation to the phenotype of β-Lg have not been found to be statistically significant, in accordance with the studies from New Zealand that did not detect an association between β-Lg phenotype and milk fatty acid composition [16, 17]. The results of these studies suggest that changes in milk composition caused by diet composition or selection practices of dairy cows might explain the differences between New Zealand and the USA [4, 17]. Therefore, further investigations will be needed to reach a definite conclusion.

Differences in milk fatty acid composition of cows with different κ-Cn and β-Lg phenotypes can suggest that genetic selection can be used for improving the texture and sensory properties of dairy products, because higher concentration of lauric acid than palmitic acid in milk fat is associated with a firmer structure and better taste of butter and cheese [4].

Another observation (Tabs. IV and V) has to do with the percentage of oleic acid present in the milk samples; the presence of this acid is considerably lower than that reported in the literature, since it is the most abundant of the unsaturated fatty acids present [15].

A logical explanation for this trend may be related to the energy level of the basic ration of cows, which can influence not only the quantity of fat produced but also the composition of lipids [7]. Consequently, it may be concluded that the lipid components of the diet may have a direct influence on the composition of the fat, while the energy level of the ration can modify the ruminal fermentative activities, which determine the availability of precursors for the synthesis of fats and the secretion of hormones which regulate lipid metabolism.

4. CONCLUSIONS

The results obtained from the current study may indicate significant profiles of fatty acid composition in the milk fat of cows with κ-casein BB and β-lactoglobulin BB. Differences in milk fatty acid composition of cows with different κ-Cn and β-Lg phenotypes could motivate researchers engaged in the field of genetic selection to try to improve the structure and the sensory and physico-chemical properties of many milk-dairy products, by selection for the B variant, particularly of κ-Cn.

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