Milk yield and quality characteristics of an endangered Italian cattle breed: the Pontremolese

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SUMMARY

The Pontremolese cow is a relic breed at risk of extinction. It reached a minimum number of heads bred in 2008 (14 heads) and more recently a slight increase was recorded in the number of individuals bred to about 70 heads. To date according to the FAO, the situation of the breed remains critical.
Given its great rusticity, the Pontremolese is used for the cow-calf breeding system in marginal areas and for the production of a typical local single-breed cheese.
This study characterizes for the first time the quality and nutritional characteristics of the Pontremolese cow's milk in order to enhance its production.
Milk samples were taken from Pontremolese cows and analyzed at 30, 60 and 90 days of lactation.
Milk was characterized by a protein content of 3.42% ± 0.344 and a fat content of. 3.5% ± 1.779. The average diameter of the fat globules was 4.63 µm ± 1.279, while the analysis of the fatty acid profile showed an average content of 69% saturated fatty acids, 31% unsaturated fatty acids, and an omega-6 / omega-3 ratio of 4.14. In addition, the milk had a higher fat desaturation index (29.25) than that reported in the literature for Bruna and Italian Friesian cattle. During the first 90 days of lactation, no statistically significant changes were observed in the quality of the milk, or in the fatty acid profile. Increases were found in the lipid content, the fat globule diameter and in the content of C15:0, C18:1 t9, C18:1 t11, of C18:3 n3 and total omega 3. Similarities in the fat and protein content were observed Reggiana cows, which are traditionally used for the production of Parmigiano Reggiano cheese. These similarities could be related to the hypothesis that traces the origin of the breed back to a variety of the Reggiana cow.

KEY WORDS

Pontremolese cow, milk quality, fatty acids, desaturase index.

INTRODUCTION

Highly selected breeds have grown in numbers at the expense of local cattle breeds, which have become endangered or extinct [1].
The excessive specialization of the livestock sector is a major cause of biodiversity losses, for instance in Europe the Friesian breed represents about 60% of livestock [2], the loss of biodiversity also leads to an impoverishment of local products.
The survival of local breeds is mainly due to their ability to adapt to environments characterized by soil and climatic conditions which are unsuitable for cosmopolitan breeds. The ability of local breeds to adapt to environmental or climate conditions is important when considering climate change. In addition, these breeds can play a socio-cultural, landscape, and natural role in promoting the region they originate from.

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In Italy there are currently 16 autochthonous cattle breeds at risk of extinction (www.associazionerare.it). The increasing demand by consumers for typical local products provides a strong incentive for the enhancement of local breeds.
The area of origin of the Pontremolese cow is Tuscany (central Italy), but in the past, the breed was also widespread in the provinces of La Spezia and Parma, and in other provinces of northern Italy. The origin of this breed has been traced back to the Parmigiana cow, a variety of the Reggiana bred in the hills. Some scholars have shown its affinity with local cattle (Bardigiana, Valtarese, Cornigliese cows) from Emilia Romagna that are now extinct and are all linked to an Iberian population that once settled on the hilly and mountainous areas of the Apennines.
The Pontremolese is a triple-aptitude breed, which showed qualities of robustness and rusticity, and in the past was used to transport marble in the Carrara quarries, or for work on the plough. This breed was also used for meat and milk production, but it decreased in number due to the competition of the more productive Alpine Brown cows (www.aia.it).

The Pontremolese is a relic breed at risk of extinction. It reached a minimum number of heads bred in 2008 (14 heads) and more recently a slight increase was recorded in the number of individuals bred to about 70 heads (www.fao.org). It is reared in small farms mainly in the Tuscan-Emilian Apennines. To date according to the FAO, the situation of the breed remains critical.

Given its great rusticity, the Pontremolese is used for the cow-calf breeding system in marginal areas. It was also included among the breeds whose meat has been allocated the Designation of Origin, «Beef of Garfagnana and Valle del Serchio» [3].

Currently Pontremolese milk is used for the production of a typical local single-breed cheese. Since the quality of milk plays an important role in all dairy products, particularly for niche ones, the aim of this study was to characterise the quality and nutritional characteristics of Pontremolese milk for the first time in order to revitalize the interest in this breed and the local cheese and to strengthen the importance of the breed in the area.

Table 1 - Chemical composition of grass hay and concentrate offered to the cows.

Components		Grass hay	Commercial concentrate
DM	g/100g	88	91.6
Crude protein	g/100g of DM	9.8	20.74
Fat		2.5	3.93
Crude fiber		33	9.06
Ash		9	6.55
Na		nd	0.22

Abbreviations: DM: dry matter; n.d.: not determined.
Trace element compounds and vitamins of the commercial feed (per kg of DM): 196 mg of iron carbonate [II], 54 mg of copper sulphate pentahydrate, 141 mg of zinc oxide, 147 mg of manganese oxide, 1.2 mg of sodium selenite, 6.5 mg of anhydrous calcium iodate, 3275 IU of vitamin A, 2183 IU of vitamin D3, 40.93 mg of vitamin E, 0.14 mg of vitamin B1, 1.09 mg Vitamin B6, 0.049 mg of vitamin B12, 109 mg of niacinamide, 76 mg of Choline Chloride, 27 mg of Betaine anhydrous.

MATERIALS AND METHODS

Animals and sampling

Individual milk samples were taken from the morning milking at 30, 60 and 90 days of lactation from seven Pontremolese cows (about 10% of the breed population) for a total of 16 milk samples (approx. 500 ml each), analysed in duplicate.

The cattle were reared in one farm and the management system was based on indoor farming, the animals were fed a traditional diet based on dry forage and concentrate (Table1); a mechanical milking system was adopted.

Chemical and physical quality of milk and somatic cell counts

Total nitrogen, caseins, dry matter and ash were determined according to the methods of the Association of Official Analytical Chemists [4].

Fat and lactose contents were analysed by infrared analysis using a fully automatic milk analyser (MilkoScan™ 7 RM; Italian Foss Electric, Padua, Italy).

The pH was assessed by the potentiometric method using a Thermo Fisher Scientific Inc. pH meter (Waltham, USA).

The somatic cell count (SCC) was evaluated by the fluor-optoelectronic method (Fossomatic Italian Foss Electric, Padova, Italy).

Milk fat extraction was performed following Rose-Gottlieb's method and methyl esters of fatty acids were prepared according to Christie [4]. All solvents and reagents were of analytical grade and were purchased from Sigma Aldrich (Milan, Italy).

A PerkinElmer Clarus 480 (PerkinElmer, Norwalk, CT, USA) equipped with a flame ionisation detector and a capillary column (ThermoScientific TR-FAME 60 m × 0.25 mm ID; film thickness 0.25 m, Fisher Scientific, Loughborough, Leicestershire, UK) was used. C5:0 was used as internal standard. The peak areas of individual FAs were identified using a FA standard injection (Food Industry FAME Mix – Restek Corporation, Bellefonte, PA, USA) and quantified as the percentage of total FAs.

The helium carrier gas flow rate was 1 mL/min. The oven temperature program level 1 was 50°C held for 5 min; level 2 was 50 to 140°C at 3°C/min, then held for 2 min; and level 3 was 140 to 240°C at 1°C/min, then held for 10 min. The injector and detector temperatures were set at 270 and 300°C, respectively.

Milk fatty acids were grouped as saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA).

To evaluate the nutritional properties, the atherogenic (AI) and thrombogenic (TI) indices were calculated as suggested by Ulbricht and Southgate [5], while the total desaturation index was calculated according to Schennink et al.[6].

Total desaturation index = (C10:1 + C12:1 + C14:1 c9 + C16:1 c9 + C18:1 c9 + CLA c9, t11)/(C10:1 + C12:1 + C14:1 c9 + C16:1 c9 + C18:1 c9 + CLA c9, t11 + C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C18:1 t11) × 100.

Morphometric analysis of milk fat globules was evaluated according to the direct method by Martini et al. [7]. The globules were grouped into three sizes: small globules (SG) with a diameter of <2 μm, medium-sized globules (MG) with a diameter from 2 to 5 μm, and large globules (LG) with a diameter of >5 μm.

Statistical analysis

Mean and standard deviation of the chemical and physical qual-

Table 2 - Chemical, physical characteristics and somatic cell count in Pontremolese cow's milk (mean and standard deviations).

-	Mean	SD
pH	6.69	0.236
Dry matter (%)	12.71	1.505
Protein (%)	3.42	0.344
Casein (%)	2.67	0.232
Fat (%)	3.5	0.779
Lactose (%)	4.61	0.573
Ash (%)	0.78	0.091
Somatic cell count *1000	154.13	129.041
Diameter of MFG (μm)	4.63	1.279
SG (%)	21.27	15.200
MG (%)	39.67	19.707
LG (%)	39.06	22.303

MFG: milk fat globules; SG: small globules; MG: medium globules; LG: large globules; SD: standard deviation.

ity of the milk and the somatic cell count were calculated.
In addition, the results were analysed using ANOVA for repeated measurements, considering the lactation period (30, 60 and 90 days) as the fixed effect and the subject as a random effect. Least significance means were compared by the t-test. Significant differences were considered at $P \leq 0.05$. Statistical analysis was carried out using JMP software [8].

RESULTS

The average milk yield produced by the morning milking of the cows was 8.00 L ± 1.60.
The average physical chemical composition of milk and the SCC are shown in Table 2. Milk had an average total protein content of 3.42% ± 0.344, while of the total proteins, on average 78% consisted of caseins (Table 2).
In terms of the lipid fraction, fat was on average 3.5% ± 1.779, and the mean diameter of the fat globules was 4.63 μm ± 1.279, in addition approximately 80% of the globules were larger than 2 microns.
Analysis of the milk fatty acid profile (Table 3) showed an average content of 69% saturated fatty acids of, 31% unsaturated fatty acids, and an omega-6 / omega-3 ratio of 4.14.
In the first 90 days of lactation, the milk samples did not show significant changes in the physico-chemical parameters (Table 4), although an increasing trend in the lipid content was found, as well as an increasing trend in the diameter of the fat globules, and in the pH and somatic cell count.
In terms of the fatty acids (Table 5), no significant differences were found in the period of the study, although increasing trends were found in the content of C15:0, C18:1 t9, C18:1 t11 of C18:3 n3 and total omega 3; while decreasing trends were found in C18:2 c9, c12 and total omega 6 and omega-6 / omega-3 ratio.

DISCUSSION

The average milk yield per milking was lower than cosmopolitan breeds, which are selected according to the milk yield, but comparable to those reported for the Reggiana [9].
The protein content, which is known to influence the milk clotting ability, together with lactose and pH, showed similar values to those in the literature for cow's milk [10,11]. The average total milk protein content detected during the period analysed was between the values reported for Italian Friesian (www.anafi.it) and Italian Brown cows (www.anarb.it) (3.35% and 3.6% respectively).
Although breed is only one of the factors to influence the quantity and quality of fat content, our results showed more similar values to those reported for the Italian Friesian (www.anafi.it) and lower than the Italian Brown (www.anarb.it) (3.7% and 4.0% respectively).
In Pontremolese milk, similarities in fat and protein contents have been reported in several autochthonous breeds from northern Italy and Reggiana cows (fat: 3.51%; protein: 3.38%) [9]. The milk compositional similarities between the Pontremolese and Reggiana breeds could be linked to origin of the breed being traced back to a variety of Reggiana traditionally used for the production of Parmigiano Reggiano cheese.
The average diameter of the fat globules was 3.5–5.5 μm, which is within the range of findings reported for cow's milk [12]. A ge-

Table 3 - Fatty acid profile, nutritional and desaturation indexes in Pontremolese cow's milk (mean and standard deviations).

Fatty acids (g/100g of total fatty acids)		Mean	SD
C10:0	Capric acid	2.61	0.809
C11:0	Undecylic acid	0.05	0.035
C12:0	Lauric acid	3.05	0.935
C13:0	Tridecylic acid	0.12	0.039
C14:0	Myristic acid	11.64	2.101
C14:1	Myristoleic acid	1.18	0.328
C15:0	Pentadecylic acid	1.55	0.364
C15:1		0.45	0.138
C16:0	Palmitic acid	32.67	4.366
C16:1 n7	Palmitoleic acid	1.84	0.482
C17:0	Margaric acid	0.83	0.246
C17:0	-	0.46	0.179
C18:0	Stearic acid	11.13	3.347
C18:1 t9	Elaidic acid	0.15	0.077
C18:1 t11	Vaccenic acid	0.95	0.407
C18:1 c9	Oleic acid	21.91	5.253
C18:2 t9,12 (n6)	-	0.4	0.145
C18:2 c9,12	Linoleic acid	1.98	0.333
C18:3 c6,9,12	γ-Linolenic acid	0.04	0.011
C18:3 c9,12,15	α-Linolenic acid	0.39	0.211
C20:0	Arachidic acid	0.22	0.095
C18.2 c9, t11	Rumenic acid	0.68	0.186
C20:1	Gondoic acid	0.25	0.063
C21:0	Heneicosylic acid	0.05	0.028
C20:2	Eicosadienoic acid	0.04	0.014
C20:3 c8,11,14	Dihomo-γ-linolenic acid	0.11	0.029
C20:4 c5,8,11,14	Arachidonic acid	0.001	0.001
C20:3 c11,14,17	Eicosatrienoic acid	0.14	0.041
C22:0	Behenic acid	0.1	0.048
C22:1	Erucic acid	0.04	0.02
C20:5 c5,8,11,14,17	Decosapentaenoic acid	0.01	0.006
C23:0	Tricosylic acid	0.04	0.009
C22:2	-	0.06	0.03
C24:0	Lignoceric acid	0.06	0.033
C24:1	Nervonic acid	0.01	0.006
C22:5 n3	Decosapentaenoic acid	0.11	0.036
C22:6 c4,7,10,13,16,19	Docosahexaenoic acid	0.01	0.006
Omega 3		0.65	0.24
Omega 6		2.54	0.395
SFA (%)		68.78	6.238
MUFA (%)		27.25	5.743
PUFA (%)		3.97	0.714
UFA/SFA ratio		0.47	0.139
Omega-6/omega-3 ratio		4.14	0.997
Atherogenic Index		2.87	0.934
Thrombogenic Index		3.40	0.880
Desaturation index		29.25	6.474

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids; SD: standard deviation.

Table 4 - Chemical, physical characteristics and somatic cell count in Pontremolese cow's milk during the first 90 days of lactation.

	Days of lactation			RMSE	P
	30	60	90		
pH	6.63	6.7	6.72	0.253	0.925
Dry matter (%)	12.83	12.46	12.91	1.601	0.546
Protein (%)	3.45	3.32	3.52	0.357	0.503
Casein (%)	2.71	2.52	2.79	0.214	0.491
Fat (%)	3.20	3.34	3.8	0.891	0.375
Lactose (%)	4.77	4.64	4.74	0.297	0.772
Ash (%)	0.75	0.70	0.78	0.091	0.850
Somatic cell count *1000	143	153.67	165.8	138.25	0.338
Diameter of MFG (μm)	4.67	4.07	5.66	1.228	0.223
SG (%)	20.26	30.03	6.42	0.305	0.078
MG (%)	43.63	37.58	33.26	20.596	0.728
LG (%)	36.10	32.39	60.32	21.156	0.187

MFG: milk fat globules; SG: small globules; MG: medium globules; LG: large globules; RMSE=root mean square error

netic component appears to exist for the average MFG size in bovine milk [13], and breed is one of the multiple factors affecting the average MFG size [12].

The milk fat globule size is an interesting trait because it affects the technological and sensory properties and nutritional quality of the milk and milk products [14,15]. In addition, milk fat globules affect the stability of the creaming rate, and their size could alter the moisture content and texture of cheese [16] .

The average somatic cell count (SCC) detected in our study was lower than the limit set by the EU regulation [17] and compatible with the good health of the udder.

An optimal somatic cell count is important both in terms of hygiene and production. In fact, cows with an SCC greater than 250000 are highly likely to be infected on at least one udder quarter, which indicates mastitis in the herd, also at a sub-clinical level [18].

From a production point of view, a high SCC is negatively correlated with milk production, lactose, fat and casein, with deleterious effects on the cheesemaking [19].

The atherogenic index (AI) and thrombogenic index (TI) take into account the effects that single FAs might have on human health and, in practice, on the probability of increasing the incidence of pathogenic phenomena such as atheroma and/or thrombus formation. Low AI and TI milk indices are considered more beneficial for health.

Results on the nutritional indices of Pontremolese cows (Table 3) showed that the atherogenic index (2.87) was between the values calculated in the literature for the Italian Friesian compared to the Italian Brown cow (2.80 and 3.03 respectively) [20,21]. On the other hand the thrombogenic index (3.40) was closer to Brown than Friesian cows (3.29 and 2.84 respectively).

The desaturation index is related to the contribution of unsaturated fatty acids in milk [21], which is linked to the dietary supply of polyunsaturated fatty acids (PUFAs) and subsequent rates of biohydrogenation in the rumen, but also to the action of the steraoyl-CoA desaturase (Δ9-desaturase) of the mammary gland. Steraoyl-CoA desaturase activity consists in desaturating saturated fatty acids by converting them into the corresponding monounsaturated fatty acids [22].

Interest in the desaturation indices derives from the fact that it generally shows higher heritability than individual fatty acids as found both in Brown [21] and in Canadian Holstein cows [23]. Desaturation indices are of interest both to evaluate the health characteristics of milk, since reducing the total intake of SFAs is recommended in the diet [24], as well as to modulate the unsaturation of fatty acids through selective strategies.

The desaturation index in Pontremolese cows (29.25) was higher than reported in the literature for Italian Brown cows (26) [21], and more similar to Canadian and Italian Friesian breeds (about 29 and 30 respectively) [23,20].

The omega-6 / omega-3 ratio was 4.14, which is within the range of cow's milk, again closer to the values in Friesian milk (3.52) than in Brown cow's milk (6.81) [21,20].

In Pontremolese milk (Table 3), the average SFA and MUFA percentages were in agreement with the literature on cow's milk [25].

Despite the presence of saturated fatty acids, milk and dairy products also provide potentially bioactive fatty acids such as vaccenic acid (VA), rumenic acid (C18:2 c9, t11) and essential fatty acids such us alpha linolenic acid (C18:3 c9, 12, 15) and linoleic acid (C18:2 c9, 12) [26].

It has been reported that the presence of C18 unsaturated fatty acids in milk may help prevent cardiovascular disease, atherosclerosis, and other chronic diseases in consumers [27].

In Pontremolese milk, the percentages of oleic, rumenic and linoleic acids were in agreement with the literature on Friesian cow's milk [20], and the average content of rumenic acid was similar to the value reported in another Italian indigenous breed, the Burlina (0.66%) [28].

As for the lactation phase, an increasing although not significant lipid content trend was found as lactation progressed. This was also followed by a similar trend in the average diameter, in agreement with the positive relation between the percentage of fat and the diameter of the milk fat globules observed in other studies [13,15].

Regarding milk fatty acid profile, its changes during the early lactation can be predictive of the energy balance of the cows and of cows suffering hyperketonemia [29].

In this study, no significant changes were found in the fatty acids profile during the 90 days of lactation even if an increasing trend

Table 5 - Fatty acid profile, nutritional and desaturation indexes in Pontremolese cow's milk during the first 90 days of lactation.

Fatty acids (g/100g of total fatty acids)		Days of lactation 30	60	90	RMSE	P
C10:0	Capric acid	2.6	2.72	2.49	0.862	0.905
C11:0	Undecylic acid	0.04	0.06	0.06	0.036	0.611
C12:0	Lauric acid	2.95	3.2	2.95	0.996	0.891
C13:0	Tridecylic acid	0.13	0.13	0.13	0.039	0.492
C14:0	Myristic acid	11.44	12.06	11.34	2.227	0.842
C14:1	Myristoleic acid	1.12	1.21	1.21	0.35	0.899
C15:0	Pentadecylic acid	1.4	1.57	1.68	0.371	0.513
C15:1		0.51	0.4	0.45	0.139	0.451
C16:0	Palmitic acid	31.12	34.36	32.19	4.434	0.482
C16:1 n7	Palmitoleic acid	1.67	1.93	1.92	0.5	0.651
C17:0	Margaric acid	0.87	0.78	0.83	0.26	0.833
C17:1	-	0.48	0.46	0.46	0.192	0.978
C18:0	Stearic acid	12.61	9.95	11.05	3.382	0.452
C18:1 t9	Elaidic acid	0.11	0.14	0.2	0.073	0.189
C18:1 t11	Vaccenic acid	0.88	0.98	0.99	0.434	0.910
C18:1 c9	Oleic acid	22.46	20.6	22.93	5.525	0.701
C18:2 t9,12 (n6)	-	0.39	0.44	0.35	0.15	0.624
C18:2 c9,12	Linoleic acid	2.09	1.94	1.93	0.349	0.714
C18:3 c6,9,12	γ-Linolenic acid	0.04	0.04	0.05	0.012	0.576
C18:3 c9,12,15	α-Linolenic acid	0.34	0.4	0.42	0.223	0.817
C20:0	Arachidic acid	0.25	0.19	0.24	0.098	0.608
C18:2 c9,t11	Rumenic acid	0.61	0.74	0.68	0.19	0.520
C20:1	Gondoic acid	0.25	0.23	0.27	0.066	0.636
C21:0	Heneicosylic acid	0.04	0.04	0.05	0.03	0.893
C20:2	Eicosadienoic acid	0.04	0.04	0.04	0.014	0.847
C20:3 c8,11,14	Dihomo-γ-linolenic acid	0.12	0.1	0.12	0.029	0.464
C20:4 c5,8,11,14	Arachidonic acid	0.001	0.001	0.001	0.001	0.950
C20:3 c11,14,17	Eicosatrienoic acid	0.15	0.14	0.13	0.042	0.611
C22:0	Behenic acid	0.11	0.09	0.11	0.05	0.601
C22:1	Erucic acid	0.04	0.03	0.05	0.019	0.319
C20:5 c5,8,11,14,17	Decosapentaenoic acid	0.01	0.01	0.01	0.006	0.739
C23:0	Tricosylic acid	0.04	0.04	0.04	0.01	0.559
C22:2	-	0.06	0.05	0.06	0.032	0.865
C24:0	Lignoceric acid	0.07	0.05	0.07	0.035	0.773
C24:1	Nervonic acid	0.01	0.01	0.01	0.006	0.359
C22:5 n3	Decosapentaenoic acid	0.1	0.1	0.12	0.037	0.679
C22:6 c4,7,10,13,16,19	Docosahexaenoic acid	0.01	0.01	0.01	0.006	0.926
Omega 3		0.61	0.66	0.69	0.256	0.900
Omega 6		2.64	2.53	2.45	0.416	0.756
SFA (%)		68.5	69.99	67.6	6.608	0.833
MUFA (%)		27.54	25.99	28.48	6.059	0.791
PUFA (%)		3.96	4.02	3.92	0.766	0.975
UFA/SFA ratio		0.47	0.44	0.49	0.148	0.849
Omega-6/omega-3 ratio		4.36	4.23	3.83	1.044	0.715
Atherogenic Index		2.75	3.13	2.70	0.951	0.732
Thrombogenic Index		3.36	3.60	3.20	0.937	0.775
Desturation index		29.57	27.94	30.54	6.848	0.818

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids; RMSE = root mean square error

was observed for C18:1 t-9.
An increasing omega 3 trend was also found and it was described by Bilal et al [23] in Canadian Holstein, which was accompanied by a decreasing trend in the omega-6: omega-3 ratio.
According to a review by Simopoulos [30], this ratio should be reduced in the diet since it is one of the most important dietary factors in the prevention of obesity, along with physical activity.

CONCLUSIONS

To the best of our knowledge this is the first study characterizing the milk of the Pontremolese relic breed. Similarities with the Reggiana breed were highlighted in the fat and protein content of Pontremolese milk, and the milk also had a higher desaturation index than Brown and Friesian cows.
The similarities observed with Reggiana milk could be linked to the hypothesis that traces the origin of the Pontremolese back to a variety of Reggiana cow, whose milk was traditionally used for the production of Parmigiano Reggiano cheese. Although further investigations are needed in this regard, the similarities observed with Reggiana milk seem to indicate that the use of this milk in dairy processing could be one way of revitalising this indigenous breed.

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