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Changes in Physical Properties of Bovine Milk from the Colostrum Period to Early Lactation

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ABSTRACT

The aim of this study was to analyze individual cows' samples from the colostrum, postcolostrum, and early lactation periods to investigate how milk composition, physical properties, stability, and suitability for processing change throughout this period. Attention was paid to the first week postpartum in which the composition of bovine mammary secretion can change markedly. Properties including pH, titratable acidity, ethanol stability (ES), rennet clotting time, and casein micelle size were analyzed, together with some compositional factors such as fat, total protein, lactose, total and ionic calcium, magnesium, citrate, phosphorus, sodium, and potassium. Total Ca (36.2 m*M*) and free ionic Ca (2.58 m*M*), Mg (5.9 m*M*), P (32.2 m*M*), and Na (24.1 m*M*) appeared to be high on d 5 postpartum, having decreased substantially over the first 5 d; they gradually decreased thereafter. The average pH on d 5 was only 6.49, compared with 6.64 at 1 mo postpartum. Stability measurements showed that the average ES on d 5 was 70% and the rennet clotting time was 12.2 min, which were significantly lower than values at later stages. A number of milk properties including ES, pH, protein content, and Ca^{2+} concentration could be useful for identifying the point of transition from colostrum to the early lactation period. Knowing the composition and physical properties of colostrum and postcolostrum secretions will help establish when such milk is suitable for processing and determine the best use for that milk.
Key words: colostrum, early lactation, casein micelle stability, ionic calcium

INTRODUCTION

Colostrum is the initial milk secreted by bovines during parturition and the first few days after birth. It provides protection to the immune system of newborn calves and provides passive immunity against pathogens. Its composition and physical properties depend on various factors including the age of the animal, number of lactation cycles, breed, diet, and diseases. Even though changes in composition and physical properties of normal bovine milk throughout lactation have been studied extensively (White and Davies, 1958a,b,c; Cerbulis and Farrell, 1976; Donnelly and Horne, 1986; Horne et al., 1986; Rodriguez et al., 2001), little is known with respect to the composition and stability of colostrum and postcolostrum milk. It has been reported that the pH of colostrum is lower (Edelsten, 1988), the specific gravity slightly higher (Haggag et al., 1991), and the immunoglobulin (IgG) content about 100 times (100 mg/mL) greater than that of normal milk (Renner et al., 1989). In the study of Klimes et al. (1986), it was found that constituents such as Ca, Mg, P, Na, and K were much greater at the first milking postpartum. Because colostrum exhibits some extreme physical properties, contamination of raw milk with colostrum could be an important issue for the dairy industry because it could affect milk processability. The elevated protein and mineral content might render milk unsuitable for certain food processing operations (such as UHT or milk powder production) and incompliant with regulations regarding milk composition for processing. In addition, increased levels of IgG can lead to reduced heat stability and weak curd formation (Feagan, 1979). According to Zawistowski and Mackinnon (1993), the presence of high levels of bovine IgG could adversely affect the human immune system.

The purpose of this study was therefore to report on the changes occurring from colostrum to early lactation in individual samples and to comment on the stability and suitability of milk for processing.

MATERIALS AND METHODS

Samples were collected from the University's farm (Reading, UK) every day (early morning milking) from 8 animals, transported to the laboratory, and stored at 4°C. All animals used in this study were Friesians; they

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had been through the dry period between October and December and did not show any health problems during the experimental period. Samples were collected on the first 5 d postpartum and then on selected days (d 15, 30, 60, and 90).

Analyses including pH, titratable acidity, ethanol stability (**ES**), rennet clotting time (**RCT**), micelle size, gross composition, and the concentrations of Na, K, Ca and Ca ions were carried out during the first 2 d to ensure that analyses reflected the composition of fresh milk.

In this study, the colostrum period was defined as the time from the first milking of the cow to d 5 of lactation; the postcolostrum period was the period from d 6 to 30 of lactation. Beyond this point, and up to 90 d, all samples were characterized as early lactation milks.

Physical Properties

ES. The stability of milk to ethanol was determined by mixing equal volumes (2 mL) of milk and a range of ethanol solutions (water/ethanol from 40 to 100%, vol/vol, at 2% intervals), and examining for the presence of clots when poured into a Petri dish. Depending upon the formation of clots, ethanol solutions of increasing or decreasing concentration were used, and the strongest concentration of ethanol that did not cause coagulation was defined as the ES. Analyses were performed at room temperature (20 ± 1°C).

RCT. Five milliliters of milk was poured in a glass test tube and maintained in a 30°C water bath. The sample was left at 30°C for 10 min, and then 13 μL of freshly prepared chymosin (Chymax Plus, Chr. Hansen, Denmark), which was diluted 1:10 with distilled water, was added. The time (min) from thorough mixing to the first sign of sudden breakdown of the film on the test tube wall was measured and defined as the RCT.

Casein Micelle Diameter. A light scattering technique was used to determine the average size of casein micelles using a Zetasizer Malvern System 3000 (Malvern Instruments Ltd., Worcestershire, UK). Samples were defatted by centrifugation at 1,410 × g for 30 min before analysis. Skim milk was diluted 50 times with double distilled water, placed in a 3-mL plastic cuvette, and analyzed.

Chemical Analyses

Fat, total protein, and lactose contents were measured using a DairyLab II Analyzer (Multispec Limited, York, UK) based on absorption of near infrared radiation at different wavelengths.

For the determination of titratable acidity (**TA**), 1 mL of phenolphthalein indicator (concentrated) was added to 10 mL of milk, and the mixture was titrated with 0.111 M NaOH to a permanent faint pink color, which was the titration end-point (pH 8.3). The TA was expressed as lactic acid concentration using the following relationship: % lactic acid (wt/vol) = 0.1 × titer volume (mL).

The concentration of P was determined using a British Standard method (British Standards, 1992). Total citrate concentration was measured following the improved method of White and Davies (1963). Total Ca and Mg concentrations were determined using the method of Murthy and Rhea (1966), employing a Pye Unicam SP9 Atomic Absorption Spectrophotometer (Pye Unicam Ltd., Cambridge, UK).

For the determination of free Ca ion concentration, a Ciba Corning 634 ISE Ca^{2+}/pH Analyser (Ciba Corning, Newbury, UK) was used. Measurements were performed at room temperature (20 ± 1°C). The operation and principle of determination have been described previously (Lin et al., 2006).

Sodium and K concentrations were measured using a 348 RapidLab (Ciba Corning Diagnostic Limited 2001). The principle of determination was the same as for the Ciba Corning 634.

Statistical Analysis

Mean values, number of determinations, and standard deviation were calculated using SPSS software (SPPS, Chicago, IL). The comparison between mean values was done using Student's t-test at a significance level of $P < 0.05$. In all other relationships, the threshold levels of $P < 0.05$, 0.01, and 0.001 were used to investigate the degree of significance.

RESULTS AND DISCUSSION

Table 1 presents average values and standard deviations (n = 8) for physical properties and composition for all individual samples collected on selected days over the first 90 d postpartum. Changes were generally substantial over the first 5 d and although less remarkable, some changes occurred between 5 and 90 d. The pH was very low initially and increased steadily thereafter. Klimes et al. (1986) found similar values for pH in the first 5 d postpartum although their pH value for the first milking was slightly higher than that found in the current study. At d 5 postpartum, the pH was 6.49 ± 0.10, which was well below the value of 6.63 measured in our previous study for a complete lactation year (Tsioulpas et al., 2007) or 6.60 measured by Edelsten (1988). According to Edelsten (1988), pH values <6.5 in milk indicate the presence of some colostrum, although low pH values could also occur due to bacterial contamina-

Table 1. Mean values and standard deviation of pH, ethanol stability (ES), rennet clotting time (RCT), titratable acidity (TA), fat, protein, lactose, ash, and casein micelle diameter of samples from the first 90 d of lactation[1]

Time (d)	pH	ES (%)	RCT (min)	TA (% lactic acid)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Casein micelle diameter (nm)
1	6.17^{a} ± 0.11	53^{a} ± 4.7	18.1^{a} ± 4.3	0.461^{a} ± 0.05	3.55^{a} ± 1.82	16.12^{a} ± 1.64	2.69^{a} ± 0.46	1.18^{a} ± 0.17	227^{a} ± 19.7
2	6.28^{a} ± 0.11	52^{a} ± 2.8	9.5^{b} ± 1.8	0.279^{b} ± 0.05	3.49^{a} ± 1.67	5.43^{b} ± 0.24	3.04^{b} ± 1.23	1.00^{b} ± 0.11	189^{b} ± 12.3
3	6.28^{a} ± 0.09	52^{a} ± 4.1	9.3^{b} ± 0.9	0.253^{b} ± 0.03	4.50^{b} ± 1.54	4.54^{c} ± 0.40	3.52^{c} ± 0.44	0.93^{c} ± 0.05	198^{b} ± 9.0
4	6.38^{b} ± 0.07	59^{b} ± 2.9	9.5^{b} ± 0.8	0.231^{bc} ± 0.02	4.26^{b} ± 2.19	4.41^{c} ± 0.31	3.82^{c} ± 0.19	0.92^{c} ± 0.08	198^{b} ± 22.5
5	6.49^{b} ± 0.10	70^{b} ± 10.2	12.2^{c} ± 3.0	0.202^{c} ± 0.02	3.89^{c} ± 1.04	4.23^{c} ± 0.24	4.15^{d} ± 0.24	0.87^{d} ± 0.04	188^{b} ± 19.6
15	6.58^{c} ± 0.05	76^{c} ± 3.8	14.0^{d} ± 2.8	0.185^{c} ± 0.03	3.66^{a} ± 1.22	4.01^{d} ± 0.43	4.32^{de} ± 0.14	0.83^{d} ± 0.04	194^{b} ± 12.8
30	6.64^{d} ± 0.10	78^{d} ± 5.7	15.8^{d} ± 4.4	0.142^{d} ± 0.02	3.72^{d} ± 0.27	3.08^{e} ± 0.19	4.54^{e} ± 0.21	0.80^{c} ± 0.06	196^{b} ± 20.5
60	6.71^{d} ± 0.01	86^{d} ± 0.0	18.3^{e} ± 5.2	0.145^{d} ± 0.00	3.95^{c} ± 2.14	2.94^{e} ± 0.19	4.61^{e} ± 0.12	0.76^{c} ± 0.00	198^{b} ± 48.1
90	6.70^{d} ± 0.11	85^{d} ± 7.5	18.9^{e} ± 3.7	0.160^{d} ± 0.02	3.51^{a} ± 0.40	3.20^{e} ± 0.22	4.70^{e} ± 0.14	0.79^{c} ± 0.01	196^{b} ± 29.2

[a–e]Means in the same column without a common letter differ significantly ($P < 0.05$).

[1]Mean values of 8 individual cows' samples.

tion. Therefore, results from the present study indicate that after 5 d, milk might still contain sufficient colostrum for it to be unsuitable for certain processing operations (taking into account that bacterial contamination has not occurred). The TA was also high and dropped to normal levels only after 2 wk. Titratable acidity measures components that exert some buffering capacity, in addition to lactic acid; these include proteins, phosphates, citrates, and carbon dioxide. In the present study, TA exhibited a good logarithmic relationship with total proteins (r = 0.95, $P < 0.001$) and with P (r = 0.91, $P < 0.001$). An inverse linear relationship with citrate (r = –0.67, $P < 0.01$) was also found.

Ethanol stability during d 1 to 4 was low for cows' milk and had only achieved 70% by d 5. Such low stability values (<60%) are common in fresh goats' milk; this has been associated with excessive sediment formation after UHT treatment and fouling of heat exchangers (Zadow et al., 1983; Prakash et al., 2007; A. Tsioulpas and M. J. Lewis, unpublished data). The average ES (± SD) in the present study was 76 ± 3.1 on d 15. According to Shew (1981), cows' milk should be stable at 74% ethanol to be suitable for UHT treatment. Thus, after 15 d, postpartum milk can be processed for UHT according to our results. It is not possible to ascertain the exact day of milk suitability, because no samples were withdrawn between d 5 and 15. Figure 1 shows that ES was low and almost constant for the first 3 d, after which it gradually increased.

According to Table 1, the average diameter of casein micelles was almost constant throughout the study except for the transition from d 1 to 2, during which time it dropped from 227 to 189 nm. The high protein (casein) and Ca (especially Ca^{2+}) concentrations on the first day might have contributed to the incorporation of more casein in the micelles, creating larger micelles.

The total protein (Table 1) in colostrum declined rapidly from 16.1 ± 1.64% at d 1 to 5.43 ± 0.24% on d 2 and then continued to decrease until 30 d, at which time it reached normal levels (3.08 ± 0.19%). The high protein content observed during early postpartum was not unusual and has been reported before (Foley et al., 1972; Sodhi et al., 1996). The data of Hadjipanayiotou (1995) showed a curved relationship with respect to CP during the first 11 d postpartum. Protein, during this period, consists of greater amounts of casein and globulins. The high proportion of Ig in colostrum provides the young calf with sufficient of this protein to develop a passive immunity system against common calfhood diseases.

There was no particular trend observed for the fat content, which varied throughout the sampling period (Table 1).

Lactose content was very low on d 1 and increased thereafter up to 60 d, at which time it reached normal levels (Table 1).

The ash content was high on the first day postpartum and then decreased gradually thereafter. Foley et al. (1972) noted that lactose contributes to one-third of the osmotic equilibrium in milk. Furthermore, they reported that during the first day postpartum, lactose

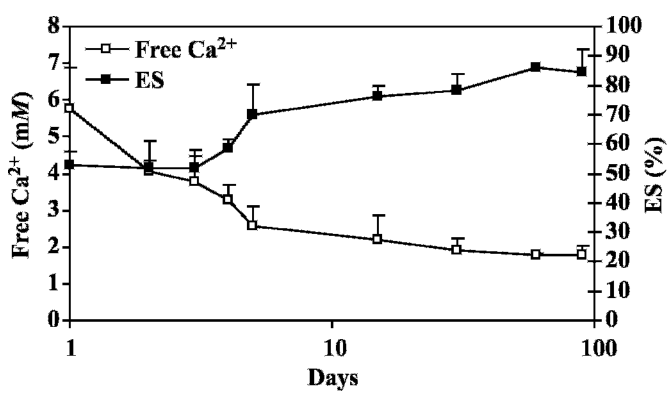

Figure 1. Changes in free Ca^{2+} concentration and ethanol stability (ES) during the first 90 d after calving. Bars represent standard deviation around the mean.

Table 2. Mean values and standard deviation of citrate, P, Ca, Mg, Ca^{2+}, Na^{+}, and K^{+} of samples from the first 90 d of lactation[1]

Time (d)	Concentration (m*M*)						
	Citrate	P	Ca	Mg	Ca^{2+}	Na^{+}	K^{+}
1	$6.62^{a} \pm 2.1$	$52.8^{a} \pm 8.6$	$54.2^{a} \pm 6.7$	$12.1^{a} \pm 1.4$	$5.75^{a} \pm 1.12$	$32.5^{a} \pm 4.7$	$40.6^{a} \pm 3.5$
2	$9.62^{b} \pm 1.1$	$40.7^{b} \pm 5.9$	$39.6^{b} \pm 7.5$	$8.3^{b} \pm 2.7$	$4.07^{b} \pm 0.84$	$30.4^{a} \pm 7.7$	$36.2^{b} \pm 7.4$
3	$11.8^{c} \pm 2.0$	$36.6^{c} \pm 2.1$	$38.7^{b} \pm 3.2$	$7.0^{c} \pm 2.8$	$3.78^{c} \pm 0.85$	$26.7^{b} \pm 3.9$	$37.9^{b} \pm 4.7$
4	$11.5^{c} \pm 2.1$	$35.7^{c} \pm 2.4$	$37.8^{b} \pm 3.8$	$6.3^{d} \pm 2.5$	$3.28^{d} \pm 0.40$	$25.8^{b} \pm 3.4$	$36.5^{b} \pm 4.3$
5	$10.2^{a} \pm 3.3$	$32.2^{d} \pm 2.3$	$36.2^{b} \pm 3.2$	$5.9^{d} \pm 2.3$	$2.58^{e} \pm 0.54$	$24.1^{b} \pm 2.8$	$35.4^{b} \pm 3.7$
15	$10.5^{ac} \pm 1.9$	$30.0^{d} \pm 4.1$	$33.5^{c} \pm 4.1$	$5.6^{d} \pm 2.1$	$2.18^{f} \pm 0.68$	$22.3^{c} \pm 4.0$	$35.5^{b} \pm 7.1$
30	$11.7^{c} \pm 2.0$	$25.1^{e} \pm 3.8$	$30.3^{c} \pm 2.2$	$5.2^{e} \pm 3.2$	$1.91^{fg} \pm 0.35$	$21.8^{c} \pm 3.3$	$36.3^{b} \pm 2.3$
60	$12.6^{d} \pm 3.7$	$23.0^{e} \pm 1.4$	$28.3^{c} \pm 3.5$	$4.7^{e} \pm 3.5$	$1.77^{g} \pm 0.04$	$15.9^{d} \pm 3.6$	$41.7^{a} \pm 1.1$
90	$12.9^{d} \pm 0.9$	$27.4^{e} \pm 1.6$	$28.6^{c} \pm 2.1$	$4.4^{e} \pm 2.1$	$1.78^{g} \pm 0.23$	$17.6^{d} \pm 2.9$	$42.4^{a} \pm 4.7$

[a–g]Means in the same column without a common letter differ significantly ($P < 0.05$).

[1]Mean values of 8 individual cows' samples.

content is inversely proportional to the K concentration, to maintain the osmotic pressure in equilibrium. In the present study, there was only a slight negative correlation ($r = -0.39$, $P < 0.05$) found between lactose and K. In addition, lactose was negatively correlated with the Na concentration ($r = -0.48$, $P < 0.05$). A correlation between Na and K was also shown ($r = -0.55$, $P < 0.05$).

Mineral composition varied during the study period as shown in Table 2. The total Ca content (m*M*) was extremely high on the first day (54.2 ± 6.7), declined sharply on the second day to 39.6 ± 7.5 and dropped to around 30.3 ± 2.2 m*M* only after 30 d. This pattern was similar for Mg and P concentrations. Free Ca^{2+} concentration (m*M*) declined sharply from 5.75 ± 1.12 to 2.58 ± 0.54 by d 5. After 30 d, it had dropped to normal levels (1.91 ± 0.31 m*M*). In another study by Sodhi et al. (1996), it was reported that the amount of ionic Ca was highly variable during the first 80 h postpartum; however, a slight decrease was apparent in the average ionic Ca content on the following day.

A high Ca ion concentration would account for low heat stability according to Parry (1974). Therefore, a value of 2.58 m*M* on d 5 postpartum, which is significantly higher than the normal values reported in the literature (2 m*M* by Holt et al., 1981; 1.88 m*M* by Tsioulpas et al., 2007), would probably render milk problematic for high heat processing. Reducing ionic calcium by various methods has been found to reduce fouling during UHT treatment of milk (Prakash et al., 2007).

It has been reported (White and Davies, 1958a,b,c; Horne and Parker, 1981; Tsioulpas et al., 2007) that ES is affected by the amount of free ionized Ca in milk. According to Figure 1, the gradual increase of ES was accompanied by a respective decrease in free Ca^{2+}. Although other factors may contribute to the changes in milk stability (pH, casein content), the amount of free Ca^{2+} is a good determinant of the ES of milk. When ES was correlated to free Ca^{2+}, an inverse nonlinear relationship with a high r-value was revealed ($r = 0.93$). A similar pattern relationship was apparent, when RCT was plotted against the amount of ionized Ca ($r = 0.80$) showing that the free Ca^{2+} may also affect the coagulation time induced by rennet.

The high concentration of Ca (and Ca ions) observed on d 1 postpartum is probably due to the high amounts of casein, which acts as a Ca carrier in milk (Holt, 2004).

Sodium concentration followed a similar pattern—it was high on d 1 and decreased thereafter. There was no particular trend regarding K concentration. Citrate remained at high levels (10.9 m*M*) during the study period compared with the average values of around 8 m*M* reported elsewhere (Walstra and Jenness, 1984; Tsioulpas et al., 2007).

Horne and Muir (1990) investigated the relationship between ES and heat stability of milk and found that these parameters differ fundamentally because they are governed by different mechanisms of coagulation. However, Chavez et al. (2004) found that individual cow milk samples stable at ethanol solutions of 78% or greater exhibited an average heat coagulation time of 23.8 min, whereas unstable samples (ES ≤72%) coagulated just before 20 min. In addition, Prakash et al. (2007) reported that occurrence of fouling on heat exchangers was significantly reduced when the ES of goat's milk was increased from about 58% to >80% by the addition of stabilizers. In this case, Ca chelating agents were used to increase ethanol stability; these reduced the Ca ion activity and changed the mineral status of milk.

Thus, milk that is collected between 5 and 15 d after lactation would not be suitable for high heat treatment but could be profitably used for products such as cheese (or yogurt), where it may be beneficial due to its lower stability and faster clotting. Supporting evidence for the latter comes from the results of Table 1 in which RCT between 5 and 15 d is lower compared with values

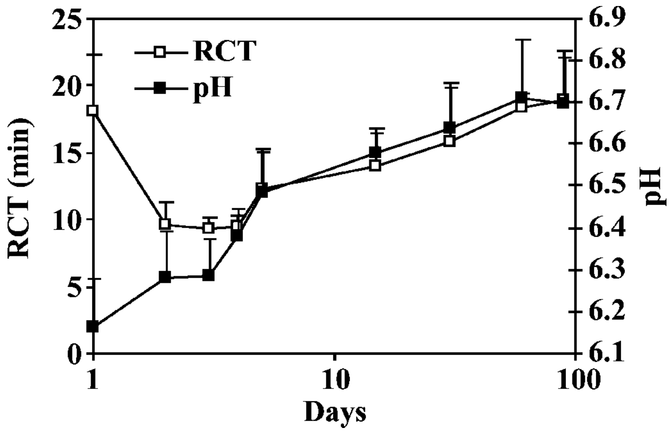

Figure 2. Changes in pH and rennet clotting time (RCT) during the first 90 d after calving. Bars represent standard deviation around the mean.

from later stages of lactation and would benefit processes requiring rapid coagulation. Colostrum samples (first 5 d postpartum), on the other hand, should not be used for any dairy applications, because their properties are different from normal, stable milk. Other possible uses as biological or medical material should be investigated. Figure 2 demonstrates the progression of RCT with time. It can be seen that RCT was high on the first day, decreased sharply, remained constant for the next 3 d, and then increased steadily. It is not surprising that RCT followed a similar pattern to pH (except on d 1) according to Figure 2, because changes in RCT can be explained by changes in pH. Madsen et al. (2004) and Fox and McSweeney (1998) reported that RCT increases when pH increases and decreases when protein content increases. This explains the gradual increase in RCT from d 4 postpartum and thereafter. The high RCT on d 1 cannot be explained; one would expect a low RCT on d 1, because pH is low and the protein level is extremely high (~16%). One suggestion is that the enzymic phase takes a long time because of the high protein to chymosin ratio, bearing in mind that coagulation does not occur until 70 to 80% of the κ-casein has been hydrolyzed.

Finally, it is interesting to note that wide variability was observed for most of the measured parameters between and within individual animals. The variation within animals can be attributed to the physiological changes that the cows are going through during the colostrum period and during the transition to the postcolostrum or early lactation periods. However, variability was also observed between animals, which can be associated with the differences in genetics, physiology, age, and number of lactation cycles of each animal. Factors including nutrition, season, or breed are not considered in the present study because all animals were of the Friesian breed, followed the same diet, and calved between October and December.

CONCLUSIONS

There are considerable changes in the physical properties of individual samples from the first day postpartum through to the early lactation period. Total Ca and Ca^{2+}, P, Mg, and Na concentrations were greater during the colostrum period compared with the postcolostrum and early lactation season. The stability of milk was very low on the first day postpartum as shown by the low pH and ES and high ionic calcium concentration. Between d 1 and d 5 there was a steady decrease in Ca^{2+} concentration, accompanied by a progressive increase in ES. Rennet clotting time was short. Ethanol stability and RCT were both influenced by the amount of ionized Ca. The ES on d 5 was 70%, which is below the threshold stability (74%) for UHT processing. Therefore, such milk could lead to processing problems, related to poor heat stability, although it would have good clotting properties. Total protein was extremely high on d 1 and dropped sharply in the next days. On the other hand, lactose content was low at the beginning of lactation and increased steadily throughout the study.

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