

MILK WITH ABNORMAL ACIDITY. VI. THE ROLE OF PHOSPHORUS CONTENT AND THE RENNET-COAGULATION PROPERTIES OF ITALIAN FRIESIAN HERD MILKS¹

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Introduction

Titrateable acidity plays a fundamental role in all phases of milk rennet-coagulation: reactivity between rennet and casein, aggregation rate of para-casein micelles and syneresis ability of the curd (1). It represents a very important parameter for the technical evaluation of the dairy-technological quality of milk. In the production of valuable cheeses (for example, the Parmigiano-Reggiano cheese), milk with abnormal acidity (especially hypoacid milk) is considered, more or less, unsuitable for cheesemaking, because of the remarkably negative consequences on the rheology of the acid-rennet curd and on the textural properties of the cheese paste (2). The pH, strictly correlated with the titrateable acidity, markedly affects the rate of hydrolysis of the k-casein by the chymosin.

There are many substances which concur in determining the natural acidity of milk. Casein and soluble phosphorus represent more than 4/5 of the titrateable acidity of the milk. In normal conditions, the contribution is almost of equal entity, while in abnormal milks, both hypoacid and hyperacid milk, such equilibrium is more or less profoundly altered because of one or both factors. One of the causes of variation in the acidity can be metabolic disorders (3, 4), which can play a very particular role, especially with reference to the acidimetric alterations arising from deficiencies and nutritional and alimentary imbalances (5, 6).

The aim of this work was to study, at the level of herd milk samples, the eventual relationship between the milk hypoacidity and the contents of casein and phosphorus, with reference also to the conditions of normal and medium-high acidity level, and evaluate their effects on milk rennet-coagulation.

Materials and methods

The study was carried out on 51 herd milk samples, equally distributed between low, medium and medium-high acidity, characterised by a normal somatic cell con-

¹ This work was supported by the experimental programme of the Emilia-Romagna region, Centro Ricerche Produzioni Animali Spa, Corso Garibaldi 42, 42100 Reggio Emilia.

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tent (Tab.1). The milk was collected in the province of Parma from small and medium size Italian Friesian dairy herds (*min.* 6 *max.* 60 lactating cows).

The screening of titratable acidity and somatic cell content, carried out to select three bulk milks with different acidity to be compared in parallel, was made monthly over the course of a year. Samplings were carried out in two cheese factories, one in the plain and one in the hill, both receiving milk from 18 dairy herds. From the first and the second cheese factory, respectively 9 and 8 homogeneous comparisons were obtained, distributed in three different seasonal periods of the year. The comparisons involved 7 different dairy herd producers of hypoacid milk in the first case, and 6 in the second one.

The following analyses were carried out on milk samples taken during morning milking from the production of the entire herd, to which 0.02% w/v sodium merthiolate was added:

- pH with potentiometer;
- Soxhlet-Henkel acidity with automatic Crison Compact D titrator;
- fat by means of mid-infrared lectures (7) with Milko-Scan 134A/B;
- total N (TN), noncasein N (NCN) and nonprotein N (NPN), respectively on milk, on pH 4.6 acid whey and on TCA 12% filtrate, by means of Kjeldahl, according to Aschaffenburg and Drewry (8); from which casein N (CN= TN – NCN), casein (CN x 6.38) and casein number (CN x 100 / TN);
- phosphorus by colorimetric method with ammonium molybdate in presence of 2.4 diaminophenol dihydrochloride according to Allen (9);
- chloride (Cl⁻) by volumetric method according to Charpentier-Volhard: clarification with zinc acetate and potassium ferrocyanide, acidification with nitric acid and re-titration of silver nitrate with 0.1 N ammonium thiocyanate (10);
- coagulation parameters (11), determined by means of Formagraph, at 35 °C on 10 ml of milk added with 0.2 ml of a rennet solution (1:19,000) diluted 1:100 with acetate buffer (pH 5.5); the technical time of analysis was 30 min (11, 12) and the following parameters were measured: r = clotting time; k₂₀ = curd firming time; a₃₀ = curd firmness measured 30 min after rennet addition;
- somatic cells (13) by Fossomatic 250 apparatus;

The statistical significance of the differences between the mean values was tested by ANOVA with SPSS program (14).

Results and discussion

Milks characterised by low (L), medium (M) and medium-high (H) titratable acidity manifested a different chemical composition and were clearly distinguishable for their rennet-coagulation properties (Tab.1). Hypoacid milk contained less casein and less phosphorus in comparison with the milk characterised by normal titratable acidity. Milk with medium-high acidity resulted the richest in phosphorus.

Differences related to phosphorus content showed a high statistical significance (mg per 100 g: 85.0 L, 86.9 M, 90.8 H; P<0.0001), but they resulted relevant only in the comparison between low and medium-high acidity milks (difference of 5.8 mg of P) and between medium and medium-high acidity milks (difference of 3.9 mg of P).

The differences related to the casein content were moderate and statistically not significant (P>0.05). These results, also in a comparison between herd milks, confirm

the fundamental role of phosphorus in determining “moderate” levels of milk hypoacidity as already observed for individual milks (15, 16).

The phosphorus content outline for all samples (Tab.1) presented analogous differences (L, M, H) also within dairy herds subdivided between plain and hill (Tab.2).

Table 1 – Characteristics of herd milks (n=51) with a different acidity level. Mean±SD

Tabella 1 – Caratteristiche di latti di massa (n=51) a diverso grado di acidità. Media±DS

Titratable acidity levels <i>Livelli acidità titolabile</i> →		Low <i>Bassa</i> (L)	Medium <i>Media</i> (M)	Medium-High <i>Medio-Alta</i> (H)	P
Herd milks <i>Latti di massa</i>	no.	17	17	17	
Titratable acidity <i>Acidità titolabile</i>	°SH	5.80 ± 0.18 ^c	6.38 ± 0.12 ^b	6.96 ± 0.30 ^a	(1)
Somatic cells <i>Cellule somatiche</i>	10 ³ /ml	319 ± 239	285 ± 184	226 ± 114	NS
Fat <i>Grasso</i>	g/100g	3.62 ± 0.29	3.75 ± 0.21	3.80 ± 0.28	NS
Casein <i>Caseina</i>	g/100g	2.40 ± 0.14	2.44 ± 0.13	2.43 ± 0.13	NS
Nonprotein N, NPN <i>Azoto non proteico</i>	mg/100g	27.4 ± 5.0	30.9 ± 4.7	29.1 ± 3.2	NS
Casein N / Total N <i>Indice di caseina</i>	%	76.91 ± 1.30	76.52 ± 1.20	76.96 ± 0.94	NS
NPN / Total N <i>NPN/N totale</i>	%	5.59 ± 0.87	6.17 ± 0.79	5.89 ± 0.65	NS
Phosphorus <i>Fosforo</i>	mg/100g	85.0 ± 3.5 ^b	86.9 ± 3.3 ^b	90.8 ± 3.1 ^a	****
Chloride, Cl ⁻ <i>Cloruri, Cl</i>	mg/100g	118.7 ± 9.6 ^a	114.3 ± 10.3 ^{ab}	106.7 ± 11.8 ^b	**
pH	–	6.73 ± 0.03 ^a	6.69 ± 0.03 ^b	6.63 ± 0.04 ^c	****
r, clotting time <i>Tempo coagulazione</i>	min	22.1 ± 3.5 ^a	19.1 ± 1.6 ^b	16.3 ± 2.3 ^c	****
k ₂₀ , curd firming time <i>Tempo rassodamento</i>	min	13.2 ± 4.9 ^a	10.8 ± 1.7 ^{ab}	9.1 ± 2.0 ^b	**
a ₃₀ , curd firmness <i>Consistenza coagulo</i>	mm	14.5 ± 7.0 ^c	21.1 ± 3.6 ^b	27.1 ± 5.3 ^a	****

(1) Differ significantly for experimental condition;

(1) *Significativamente diversi per condizione sperimentale;*

a, b, c, differ for P<0.05

a, b, c, differiscono per P<0,05

NS, P>0.05; **, P≤0.01; ****, P≤0.0001

Titrateable acidity was highly positively correlated ($r = +0.71$; $P < 0.0001$) with milk phosphorus content (Tab. 3), a result that is confirmed also by other studies (16, 17) conducted at the level of individual milks.

Chloride content manifested a diametrically opposite trend to that of phosphorus: its values were higher in hypoacid milk (mg per 100g: 118.7 L, 114.3 M, 106.7 H; $P < 0.01$) (Tab.1). In fact, chloride content was negatively correlated both with acidity ($r = -0.52$; $P < 0.001$) and with phosphorus content ($r = -0.57$; $P < 0.0001$), as observed in a previous research (18) (Tab.3).

Results illustrated in figure 1, even if significant, do not show such an outline (19) to depict an evident direct relationship between abnormal acidimetric conditions in milk and substantial imbalances in the nutritional-metabolic *status* of the dairy cows.

In any case, the role of alimentary factors in determining milk acidity (1, 3, 20) appears very complex, such as to allow for the supposition of the involvement of general metabolic conditions, which, even if moderately abnormal, could induce, especially over a long period of time, alterations in the acid-base equilibria (6), which, in turn, could reverberate on milk salt equilibria and, consequently, also on its rennet-coagulation properties, as the present research can confirm.

Hypoacid milk, characterised by a higher pH, manifested considerably longer clotting times and curd firming times. The same milk, at 30 min after rennet addition, supplied a curd with very low firmness as compared to that of milk with normal titrateable acidity (Tab.1).

In fact, different clotting time (r in min: 22.1 L, 19.1 M, 16.3 H; $P < 0.0001$) and curd firming time (k_{20} in min: 13.2 L, 10.8 M, 9.1 H; $P < 0.01$) were registered. Curd firmness, also, resulted significantly different for the three levels of titrateable acidity (a_{30} in min: 14.5 L, 21.1 M, 27.1 H; $P < 0.0001$), as well as observed for the clotting time.

In effect, both clotting time (-0.74 ; $P < 0.0001$), and curd firming time (-0.50 ; $P < 0.001$), and curd firmness measured 30 min after rennet addition (0.74 ; $P < 0.0001$) were strictly correlated to the milk acidity value.

Conclusions

The acidity level of herd milk samples appears significantly affected by the phosphorus content; while there is no evidence of important variations regarding casein content. Low acidity milk contains less phosphorus, both in comparison to medium acidity milk and, especially, in comparison to medium-high acidity milk.

The same milks, however, even if with a similar and normal content of somatic cells, are characterised by a significantly different chloride content in relation to the three titrateable acidity levels. Low acidity milk has a higher chloride content, both in comparison to the medium acidity one, and, even more so, in comparison to the medium-high acidity one.

Correlations between titrateable acidity and phosphorus ($r = 0.71$) and between phosphorus and chloride ($r = -0.57$) are high and statistically significant ($P < 0.0001$). The same correlations can be considered such as to posit an eventual relationship with alterations in the metabolic *status* of the cow, with particular reference to blood acid-base equilibrium.

Herd milk, characterised by a low titratable acidity, shows significantly higher pH values and coagulates in a significantly longer time, both in comparison to medium and to medium-high acidity milk.

Keywords: Italian Friesian cattle, herd milk, phosphorus, titratable acidity, rennet-coagulation

Parole chiave: Frisona italiana, latte di massa, fosforo, acidità, coagulazione pre-samica

Table 2 – Characteristics of herd milks, with a different acidity level, produced in plain and hill herds. Mean values.

Tabella 2 – Caratteristiche di latti di massa, a diverso grado di acidità, prodotti negli allevamenti di pianura e di collina. Valori medi.

Titratable acidity levels <i>Livelli acidità titolabile</i> →		Plain - <i>Pianura</i>			Hill - <i>Collina</i>		
		L	M	H	L	M	H
Herd milks <i>Latti di massa</i>	no.	9	9	9	8	8	8
Titratable acidity <i>Acidità titolabile</i>	°SH	5.90	6.42	6.98	5.68	6.36	6.94
Somatic cells <i>Cellule somatiche</i>	10 ³ /ml	446	375	239	177	184	211
Fat <i>Grasso</i>	g/100g	3.64	3.81	3.79	3.60	3.69	3.81
Casein <i>Caseina</i>	g/100g	2.40	2.44	2.44	2.41	2.43	2.41
Nonprotein N, NPN <i>Azoto non proteico</i>	mg/100g	30.2	32.4	30.7	24.3	29.1	27.3
Casein N / Total N <i>Indice di caseina</i>	%	76.26	76.22	76.64	77.65	76.86	77.33
NPN / Total N <i>NPN / N totale</i>	%	6.12	6.44	6.15	4.99	5.88	5.59
Phosphorus <i>Fosforo</i>	mg/100g	86.8	88.4	92.3	83.0	85.1	89.1
Chloride, Cl <i>Cloruri, Cl</i>	mg/100g	114.9	111.2	107.8	123.0	117.8	105.5
pH	–	6.72	6.68	6.63	6.74	6.69	6.65
r, clotting time <i>Tempo coagulazione</i>	min	21.2	18.8	16.6	23.0	19.4	16.0
k ₂₀ , curd firming time <i>Tempo rassodamento</i>	min	12.6	10.6	9.0	13.9	11.0	9.1
a ₃₀ , curd firmness <i>Consistenza coagulo</i>	mm	15.7	21.9	27.4	13.1	20.3	26.6

Table 3 – Significance of correlations between the characteristics of herd milks (n=51) with a different acidity level. Correlation coefficients.

Tabella 3 – Significatività delle correlazioni tra le caratteristiche di latti di massa (n=51) a diverso grado di acidità. Coefficienti di correlazione.

	Casein	Phosphorus	Chloride	pH	r	k ₂₀	a ₃₀
Acidity <i>Acidità</i>	0.07	0.71 ****	-0.52 ***	-0.85 ****	-0.74 ****	-0.50 ***	0.74 ****
Casein <i>Caseina</i>	1	0.23	0.32 *	0.14	0.10	-0.32 *	0.18
Phosphorus <i>Fosforo</i>		1	-0.57 ****	-0.62 ****	-0.56 ****	-0.49 ***	0.64 ****
Chloride <i>Cloruri</i>			1	0.46 ***	0.44 **	0.35 *	-0.41 **
pH				1	0.78 ****	0.45 ***	-0.73 ****
r					1	0.66 ****	-0.89 ****
k ₂₀						1	-0.81 ****
a ₃₀							1

* P≤0.05; ** P≤0.01; *** P≤0.001; **** P≤0.0001

SUMMARY - The relationships between casein and phosphorus contents and titratable acidity were studied. The research was carried out on 51 herd milk samples characterised by low (L), medium (M) and medium-high (H) titratable acidity. Low acidity milk presented lowest casein and phosphorus contents. Differences in phosphorus content were statistically significant (mg per 100 g of milk: 85.0±3.5 L; 86.9±3.3 M; 90.8±3.1 H; P<0.0001). Chloride content, instead, was highest in the low acidity milk (mg of Cl⁻: 118.7±9.6 L; 114.3±10.3 M; 106.7±11.8 H; P<0.01). Titratable acidity resulted positively correlated with phosphorus content (r = 0.71; P<0.0001) and negatively with chloride content (r = -0.52; P<0.001). Milk characterised by a low acidity had a markedly longer clotting time (min: 22.1 L, 19.1 M, 16.3 H; P<0.0001). In effect, the negative correlation between clotting time and milk acidity resulted very high (r = -0.74; P<0.0001).

RIASSUNTO - *Il latte ad acidità anomala. VI. Il ruolo del fosforo e le proprietà di coagulazione presamica di campioni di latte di massa di vacche di razza Frisone italiana.*

Sono stati studiati i rapporti tra i contenuti di caseina e di fosforo e l'acidità titolabile del latte. L'indagine è stata condotta su 51 campioni di latte di massa di singoli allevamenti caratterizzati da bassa (L), media (M) e medio-alta (H) acidità titolabile. Il latte a bassa acidità è risultato più povero di caseina e di fosforo. Le differenze riguardanti il fosforo sono risultate statisticamente significative (mg P per 100 g latte: 85,0 L; 86,9 M; 90,8 H; P<0,0001). Per contro, il latte a bassa acidità è risultato più ricco di cloruri (mg Cl⁻ per 100 g latte: 118,7 L; 114,3 M; 106,7 H; P<0,01). L'acidità titolabile è risultata correlata positivamente con il contenuto di fosforo (r = 0,71; P<0,0001) e negativamente con il contenuto di cloruri (r = -0,52; P<0,001). Il

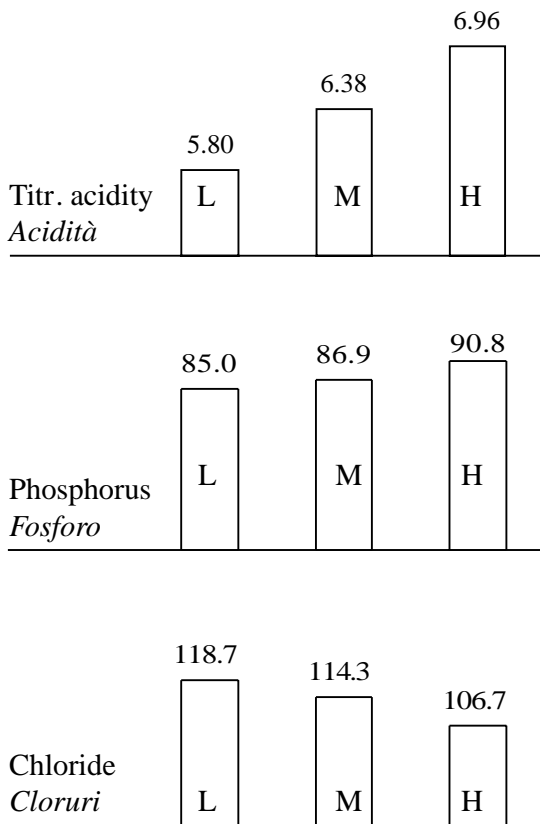


Figure 1 - Titratable acidity ($^{\circ}\text{SH}$) and phosphorus (mg /100 g) and chloride contents (mg of Cl^- / 100 g) of herd milks (n=51) with a different acidity level: L = low; M = medium; H = medium- high.

Figura 1 - Acidità titolabile ($^{\circ}\text{SH}$) e contenuti di fosforo (mg/100g) e di cloruri (mg di Cl^- / 100 g) di latte di massa (n= 51) a diverso grado di acidità titolabile: L = bassa; M = media; H = medio- alta.

latte caratterizzato da bassa acidità titolabile ha manifestato tempi di coagulazione nettamente più lunghi (min: 22,1 L; 19,1 M; 16,3 H; $P < 0,0001$). In effetti, la correlazione negativa tra tempo di coagulazione e acidità del latte è risultata molto elevata ($r = -0,74$; $P < 0,0001$).

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