

Original article

## Chemical composition and coagulation properties of Modicana and Holstein cows' milk

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**Abstract** — The chemical composition and clotting properties of milk from 45 Modicana (Mo) and Holstein (Ho) cows were assessed over the winter (December and January) and spring periods (March–April). The cows came from 4 farms of the Ragusa region (Italy). Considerable differences in milk yield, milk composition and clotting properties were noted between breeds. During the winter and spring periods, Mo and Ho produced an average 15.4 and 35.8 kg·day<sup>-1</sup> of milk, respectively. The Mo milk contained significantly more fat (+5.8 g·kg<sup>-1</sup>), nitrogen (+5.9 g·kg<sup>-1</sup>) and lactose (+1.9 g·kg<sup>-1</sup>). The casein/true protein ratio was also higher in Mo milk (+3.5%) and the plasmin content was 87% lower. The fatty acid composition was the same in both breeds. The pH of the Mo milk was lower (–0.07), coagulation time and curd firming rate were quicker (32 and 53% respectively) and the curd was firmer (+50%). The results obtained during the spring period were very close to those of the winter period. For a same nitrogen content, the Mo milk produced a 16% firmer curd than the Ho milk, probably due to its higher casein/protein ratio and its higher content of casein κ variant B (75 and 28% for Mo and Ho cows respectively).

**milk / Holstein and Modicana breeds / chemical composition/ clotting properties**

**Résumé** — **Composition chimique et aptitude à la coagulation du lait des races bovines Modicana et Holstein.** La production, la composition chimique et l'aptitude à la coagulation du lait de 45 vaches de race Modicana (Mo) et Holstein (Ho) vêlant à partir du mois de septembre ont été contrôlées en hiver (décembre et janvier) et au printemps (mars et avril). Les animaux provenaient de 4 élevages de Ragusa en Italie (Sicile). Des différences considérables ont été observées entre les races pour la production laitière, la composition chimique et l'aptitude à la coagulation du lait. En moyenne, au cours de l'hiver et du printemps, les Mo et Ho ont produit respectivement 15,4 et 35,8 kg·jour<sup>-1</sup> de lait. Le lait Mo a été significativement plus riche en matières grasses (+5,8 g·kg<sup>-1</sup>), en matières azotées totales (+5,9 g·kg<sup>-1</sup>) et en lactose (+1,9 g·kg<sup>-1</sup>). Le rapport caséines/proteines,

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mesuré sur les laits de 2 troupeaux, a été nettement plus élevé pour les Mo (+3,5 %) et la teneur en plasmine plus faible de 87 %. La composition des acides gras a été similaire entre les deux races. Le pH des laits Mo été plus faible (-0.07), la coagulation et le raffermissement du caillé plus rapides (respectivement 32 et 53 %) et le caillé obtenu plus ferme (+50 %). Pour un même taux protéique, le lait Mo a conduit à un gel plus ferme de 16 % par rapport au lait Ho, vraisemblablement en raison de son rapport caséine/protéines plus élevé et de sa plus grande richesse en variant B de la caséine  $\kappa$  (75 et 28 % respectivement pour les vaches Mo et Ho). Comparativement aux différences observées entre les laits des 2 races, les différences observées entre périodes ont été très faibles et nous n'avons pas observé d'interactions entre la race et la période.

#### lait / races Holstein et Modicana / composition chimique / aptitude à la coagulation

## 1. INTRODUCTION

Research for always higher milk yield has, in the last decades, promoted the strong expansion of a very small number of dairy cattle breeds (Holstein in particular) and the rapid decrease of a variety of less productive breeds. This evolution is not without implications for the future because it may induce the extinction of local breeds. These breeds may nevertheless offer interesting characteristics, especially in rural and difficult farming areas. This situation is particularly pronounced in Sicily, where the local breed, Modicana, is gradually being replaced by the Holstein breed. The Modicana cow, coloured red, originates from the Ragusa region. Its characteristics have been described by some authors [11]. The Modicana breed is used both for meat production, through an industrial cross-breed with the Limousin or Charolais, and for milk production used to make a local cheese called "Ragusano" with a Protected Denomination of Origin. Because "Ragusano" cheese can be produced either with milk from Modicana or Holstein cows, it is crucial to better characterise the cheese-making properties of their milk. Modicana cows are appreciated by breeders for their maternal qualities and for their adaptability to adverse conditions, dietary in particular. This breed, which now counts 4500 heads, produces an average 3000 kg of milk for 262 lactation days [1]. In comparison with Holstein milk,

Modicana milk is characterised by a higher protein content and a high frequency of casein  $\kappa$  variant B [6]. The milk fat composition [7], the mineral content and coagulation properties [13, 15] of Modicana milk have also been documented. Other characteristics such as the content in casein or proteolytic enzymes have never been explored.

The aim of this study was to describe the chemical composition and coagulation properties of milk produced by Modicana and Holstein cows reared on local farms.

## 2. MATERIALS AND METHODS

### 2.1. Animals and feeding

Twenty-three Modicana (Mo) and 24 Holstein (Ho) cows from 4 farms of the Ragusa region were used for this study (Tab. I). The animals were selected according to calving dates (September–January) and high production levels for multiparous cows. The trial started on 23 September 1997. Thirteen of the 47 cows were primiparous and 34 were multiparous. The cows were milked twice a day between 06.00/07.00 and 17.00/18.00 on all farms. Milking of Modicana cows was primed by their calf for approximately one minute. The calf was returned to suckling at the end of milking. For each breed, one of the farms used a winter forage composed exclusively of hay (55 and 40% DM of concentrate for the Ho and

**Table I.** Main characteristics of the 4 cattle farms.

Breed	Ho	Ho	Mo	Mo
Farm	1	2	1	2
Overall number of cows	100	30	30	50
Number of cows included in the trial	12	12	11	12
Total days of lactation	286	270	230	230
Average lactation number	3.8	2.4	2.9	2.5
Mean live weight (kg)	600	610	540	550

Mo farms, respectively), and the other a winter forage composed of hay and maize silage (20% hay, 25% maize silage and 55% concentrate for the Ho farm and 50% hay, 20% maize silage and 30% concentrate for the Mo farm). The nutritional value of the diets used was, for the energy supply, 0.91 and 0.82 UFL·kg<sup>-1</sup> DM on Ho farms and 0.75 and 0.72 UFL·kg<sup>-1</sup> DM on Mo farms and, for nitrogen supply, 107 and 85 g PDI·kg<sup>-1</sup> DM in Ho farms and 73 and 80 g PDI·kg<sup>-1</sup> DM in Mo farms. On the Ho farm with maize silage, feed was given throughout the year as a total mixed ration, and the cows were never turned out to graze. On the other farms, feeds were given separately and from December onwards, the animals were taken outside but grass intake was negligible until the end of February (grass growth period) where the amounts of winter feeds were gradually reduced to about 50%.

## 2.2. Measurements

Milk yield was monitored individually once a month during the morning and evening milkings. Experimental samples of milk were collected in December, January, March and April. They were composed of a mixture (50/50) of the entire evening milking (stored at 4 °C overnight without any preservative) and the morning milking. They were shipped in an insulated package to the laboratory and were analysed within 4 hours

following reception; fat, total nitrogen and lactose contents were assessed using a Milkoscan instrument (Foss Electric, Hillerød, Denmark). Milk urea was measured by spectrophotometry (Lambda 20 Perkin-Elmer, Uberlingen, Germany), following protein depletion with 0.3 mol trichloroacetic acid (Sigma Diagnostics, St-Louis, Mo, USA). The pH was measured by potentiometry using an Orion EA 940 (Orion Research, Boston, MA). The somatic cell count was performed using a Fosomatic instrument (Foss Electric, Hillerød, Denmark). Rennet coagulation time (in minutes), curd firming rate ( $k_{20}$  in minutes) and curd firmness, measured 30 minutes after the addition of rennet ( $a_{30}$  in mm) and after a firming time equal to the coagulation time ( $a_r$  in mm), were measured with a Formagraph (Foss Electric, Hillerød, Denmark), in 10 mL of milk at the initial pH and 35 °C with a 200 µL dose of rennet (title 1:18.000).

At the beginning (weeks 3–6), in the middle and at the end of lactation, 2 samples were immediately frozen. These samples were used to titrate calcium by atomic absorption spectrophotometry [10], and to analyse fatty acid composition by high resolution gas chromatography (Shimadzu mod. GC 17 A Tokyo 101, Japan) after extraction of neutral lipids and preparation of methyl esters after acid transesterification. For each breed, the samples collected on the farm using only hay as a winter forage were also used to determine protein

genetic variants ( $\alpha$ s1-,  $\alpha$ s2-,  $\beta$ - and  $\kappa$ -CN and  $\beta$ -lg) by isoelectric focusing [21], total N, non-protein N and casein content by the Rowland [18] method. Plasmin and plasminogen-derived activities were determined using a modification of the method of Rollema et al. [17].

A sample of each feed used was collected once during the winter for chemical composition analysis (dry matter, organic matter, crude fibre, total nitrogen content). The nutritional value of the feeds was estimated from the results of these analyses according to the formulas used in the "PREVALIM" software [5].

### 2.3. Data analysis

Data from December and January for the winter period and March and April for the spring period were averaged, except for calcium, fatty acids, casein/true protein ratio and plasmin and plasminogen (a single sample collected at each period). All data except casein, plasmin and plasminogen were processed by analysis of variance (GLM procedure, SAS) [19] by introducing the breed, period, herd within breed and interaction breed\*period into the model. Data for casein, plasmin and plasminogen were processed by introducing the breed, period and interaction breed\*period into the model.

## 3. RESULTS

The main health disorder observed during the trial was mastitis. Two cows from one herd (Mo-No. 2) were withdrawn from the trial following mastitis occurrence in early lactation. The final population was therefore 45 cows.

### 3.1. Chemical composition of milk

Average milk yield over the entire lactation period was 3468 kg over 230 days

and 9563 kg over 278 days of lactation in Mo and Ho cows, respectively. The lactation curve of Mo cows was flatter than that of Ho cows. The average values of fat and total nitrogen contents over the whole lactation were 35.0 g·kg<sup>-1</sup> and 30.6 g·kg<sup>-1</sup> in Ho cows and 42.5 g·kg<sup>-1</sup> and 36.9 g·kg<sup>-1</sup> in Mo cows, respectively.

On average during the 2 periods (Tab. II) Mo cows' milk contained significantly more fat (+ 5.8 g·kg<sup>-1</sup>;  $P < 0.001$ ), more total nitrogen (+ 5.9 g·kg<sup>-1</sup>;  $P < 0.001$ ) and more lactose (+ 1.9 g·kg<sup>-1</sup>,  $P < 0.001$ ). The milk somatic cell count, urea and calcium content as well as the composition of fatty acids were similar for the milk from both breeds ( $P > 0.05$ ). On a limited number of samples, Mo cow's milk also had a significantly higher casein/true protein ratio (+ 3.5%,  $P < 0.01$ ) and a very lower plasmin content ( $P < 0.001$ ). The plasminogen content of Mo milk was also lower although the differences were not statistically significant. In addition, the analyses realised on the cows used for casein titration revealed, respectively for Mo and Ho cows, a frequency of the B variant of 75 and 28% for  $\kappa$ -casein and 81 and 61% for  $\beta$ -lactoglobulin.

Comparatively to the influence of the breed, the influence of the period of the year was very small (Tab. II). The total nitrogen content of spring milk (March/April) was significantly higher by +1.4 g·kg<sup>-1</sup> ( $P < 0.01$ ), especially for Mo cows and the spring milk also had a lower lactose content (-0.9 g·kg<sup>-1</sup>,  $P < 0.05$ ).

### 3.2. Coagulation properties

Considerable differences in coagulation properties were noted in favour of Mo cows. On average during the winter and the spring periods, Mo cows had more acidic milk (lower pH from 0.07;  $P < 0.01$ ) and a more rapid coagulation time and curd firming rate, (32% and 53%, respectively). Mo milk also produced a curd that was 55 and 50% firmer respectively thirty minutes after rennet

**Table II.** Yield, chemical composition and coagulation properties of milk from Modicana (Mo) and Holstein (Ho) cows during two periods (winter-spring).

	Breed		Breed	Period	Breed* Period	RSD <sup>3</sup>
	Mo	Ho				
<b>Chemical composition</b>						
Milk yield (kg·j <sup>-1</sup> )	15.4	35.8	***	ns	ns	5.1
Fat content (g·kg <sup>-1</sup> )	44.3	38.5	***	ns	ns	6.4
Total nitrogen content (g·kg <sup>-1</sup> )	37.9	32.0	***	**	**	2.5
Lactose (g·kg <sup>-1</sup> )	53.3	51.4	***	*	ns	1.9
Somatic cell count (log·mL <sup>-1</sup> )	5.47	5.34	ns	ns	ns	0.37
Urea (mg·100 mL <sup>-1</sup> )	22.1	21.5	ns	ns	ns	7.6
Calcium (g·kg <sup>-1</sup> )	1.24	1.14	ns	ns	ns	0.10
Casein/True protein ratio (%)	81.0	77.5	**	ns	ns	3.2
Plasmin (arbitrary unit)	3.1	5.8	***	ns	ns	1.9
Plasminogen (arbitrary unit)	19.9	23.6	ns	ns	ns	8.5
Short chain fatty acids (%)	10.0	9.7	ns	ns	ns	0.7
Medium chain fatty acids (%)	45.4	46.2	ns	ns	ns	2.3
Long chain fatty acids (%)	36.4	37.7	ns	ns	ns	2.7
Unsaturated fatty acids (%)	30.0	29.7	ns	ns	ns	1.9
<b>Coagulation properties</b>						
pH	6.57	6.64	***	ns	ns	0.06
Coagulation time (min)	10.5	15.4	***	ns	**	2.8
Curd firming rate (min)	3.7	7.9	***	ns	ns	1.6
<sup>1</sup> Curd firmness (mm)	50.0	32.2	***	ns	ns	6.6
<sup>2</sup> Curd firmness (mm)	42.8	28.5	***	ns	*	4.7

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns:  $P < 0.05$ .

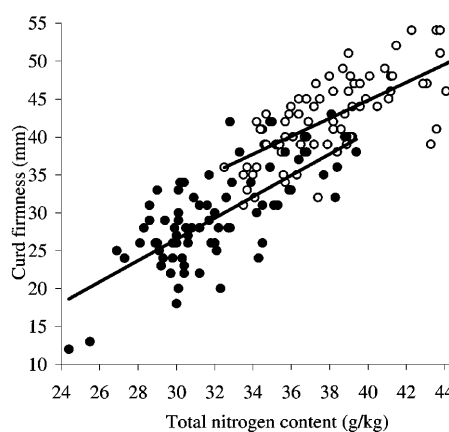
<sup>1</sup> Measured 30 minutes after rennet addition.

<sup>2</sup> Measured after a firming time equal to clotting time.

<sup>3</sup> Residual standard deviation.

addition and after a firming period equal to the coagulation time. Milk coagulation parameters were identical in the winter and spring periods and there was no significant interaction between breed and period, except for milk coagulation time that decreased in the spring only for Ho cows.

Curd firmness assessed after a curd firming time equal to the coagulation time was positively linked to total nitrogen contents ( $r = 0.86$ ;  $P < 0.001$ ). For a same nitrogen content, curd firmness remained 16% higher in Mo cows (Fig. 1). When taking nitrogen content into consideration as a covariable in the linear model, the inter-breed differences in curd firmness remained statistically significant ( $P < 0.01$ ); 33.3 and 38.6 mm in Ho and Mo cows, respectively.



**Figure 1.** Influence of total nitrogen content on curd firmness according to breed: Holstein (●) and Modicana (○).

#### 4. DISCUSSION

This study has revealed the very specific characteristics of Modicana cows' milk in relation to Holstein cows. The differences in fat and nitrogen contents were particularly marked but they were consistent with those already described [13]. The fat content results for Mo cow's milk were additionally underrated since the end-milking part of milk, which is the richest in fat, was not sampled because of the use of the calf for milking. The higher casein/true protein ratio of Modicana milk may be explained by its higher frequency of the B variant of  $\beta$ -lactoglobulin and/or  $\kappa$ -casein as already shown intra-breed [8]. The higher frequency of the B variant of  $\beta$ -lactoglobulin and  $\kappa$ -casein for Modicana cows obtained in this trial for a small number of cows, is consistent with other results for Modicana cows [6, 13]. The higher plasmin content of Holstein milk could also be responsible for the proteolysis of casein and may therefore explain one part of the difference of the casein/true protein ratio.

To our knowledge, the very low plasmin content of Modicana's milk has never been observed before. It is well known that certain factors such as mastitis, udder health disease or a high number or stage of lactation increase the plasmin level [4], but these factors were similar for the two breeds. Bastian et al. [3] found that casein interferes with plasmin assays that utilise synthetic substrates. This interference can be avoided by reducing the casein/substrate ratio. The assay used for plasmin and plasminogen-derived activities determination in this study was linear for a range of casein concentrations between 20 and 40 g·L<sup>-1</sup>. Therefore, in our case, the differences observed in plasmin activities between Mo and Ho cannot be explained by differences in the casein concentration. However, differences between breeds have already been observed by Schaar [20] who compared Jersey and Friesian cows and showed a lower content for the less productive cows as we did.

The higher lactose content of Modicana milk, already noted by Marletta [13], is surprising. It is indeed commonly accepted that lactose content is inversely proportional to the contents of other dissolved elements (minerals in particular) because of the sustained osmotic pressure [2] and generally inversely proportional to fat and protein contents. Such was not the case with available elements in this study (fat, nitrogen and total calcium contents) but other mineral elements important for osmotic pressure (Na, K, Cl...) were not analysed.

The results obtained in this study confirm the better coagulation properties of milk from Modicana cows. The shorter coagulation time can be explained by the lower pH of the milk, as often observed [16]. The greater curd firmness of Mo cows' milk is essentially due to its higher nitrogen content, as observed by a number of authors [16, 22] and confirmed herein. The higher curd firmness of Modicana milk with the same nitrogen content could be attributed firstly to the polymorphism of casein  $\kappa$ , as evidenced in individual [9] or mixed milks [14]. Indeed, it is a known fact that the casein  $\kappa$  variant B is sharply more frequent in Modicana cows [6, 13] than in Holstein cows [12] and it has been confirmed in this trial. Secondly, the higher casein/true protein ratio of Modicana milk may also contribute to explaining the differences in curd firmness observed with the same nitrogen content [16].

#### 5. CONCLUSIONS

This study has shown the existence of considerable breed-dependent differences in the chemical composition and coagulation properties of milk. The milk from Modicana cows is technologically much more suitable for coagulation than Holstein milk. For the (Ragusano) cheese-making process with the Protected Denomination of Origin, these characteristics may be highly valuable and need to be confirmed by studies involving the characteristics of ripened cheeses.

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