J. Dairy Sci. 90:3986–3992
doi:10.3168/jds.2006-627

Milk Coagulation Ability of Five Dairy Cattle Breeds

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ABSTRACT

Samples of herd milk (506) were analyzed to assess sources of variation for milk coagulation properties (MCP) for 5 different dairy cattle breeds. Data were recorded in 55 single-breed dairy herds in the Trento province, a mountain area in northeast Italy. The 5 cattle breeds were Holstein-Friesian (8 herds), Brown Swiss (16 herds), Simmental (10 herds), Rendena (13 herds), and Alpine Gray (8 herds). Herd milk samples were analyzed for the MCP traits, milk rennet coagulation time (RCT), curd-firming time, and curd firmness (a_{30}), as well as protein and fat percentages, somatic cell count, Soxhlet-Henkel acidity, and bacterial count. An ANOVA was performed to study the effect of breed, herd within breed, DIM, month of lactation, protein and fat percentages, somatic cell score, titratable acidity, and log bacterial count within breed on MCP. Breed was the most important source of variation. In particular, the Rendena breed showed the best MCP traits at 13.5 min and 27.0 mm for RCT and a_{30}, respectively. The Holstein-Friesian breed had the worst coagulation properties at 18.0 min and 17.5 mm for RCT and a_{30}, respectively. The other 3 breeds showed intermediate coagulation properties. The RCT values were better at the beginning of lactation, whereas RCT and a_{30} values were better in September and October (14.3 min and 25.7 mm, respectively). Among the composition traits, only the titratable acidity affected MCP traits of herd milk positively.
Key words: milk coagulation ability, dairy cattle breed, herd milk

INTRODUCTION

The structure of Italian dairy cattle herds has changed radically in the last several decades. The most important changes have been the progressive concentration and specialization of dairy herds. In Italy at present, more than 70% of Italian milk in Italy is used for cheese production (Osservatorio del Latte, 2003) and the 80% of that is used for typical Italian products (e.g., Parmiggiano-Reggiano and Grana Padano).

In the plain area, large herds are mainly Holstein-Friesian (**HF**) with high milk yield used for the production of large amounts of high priced cheeses like the Grana Padano, Parmigiano-Reggiano, and Gorgonzola (Osservatorio del Latte, 2003). In the northern Italian mountain areas, milk production is traditionally based on small farms that also use pastures and local dual-purpose breeds (Alpine Brown, Simmental (**S**), Alpine Gray (**AG**), Rendena (**R**), and Red Pied Valdostana) that are characterized by a medium level of production, as well as better functional and conformation traits. In the Italian Alps, there are a dozen of traditional local cheeses with strong local demand and a reasonable price. In addition, in the Trento mountain region, there is a tendency toward specialized herds. Brown Swiss (**BS**) replaced Alpine Brown cows, and HF ranks second in number of cows.

Local breeds can be considered cultural assets because of their role in local traditions and because they have often played a central part in the social life of rural populations. Local breeds can also be linked to local culture because they contribute to the preservation of ancient local traditions and food products. To evaluate these diminishing local breeds that historically produced niche cheese products, the study of their qualitative traits will be important.

Currently the dairy industry plays a key role in the economic development of the Trento province, a mountain area in the northeastern Italian Alps. Local cheese production is quite differentiated. Besides Grana cheese (3,500 tons per year), other important niche cheese products such as Spressa, Vezzena, Puzzone di Moena, and Casolet have always been made. Increasingly, consumer choice and decision-making depends on product image before purchasing and during consumption. This perception of a product is referred to as quality expectation (Kupiec and Revell, 1998). Quality expectations are themselves affected by several factors including product provenance.

In the meantime, the small dairies of the mountain regions have observed a decrease in the coagulation ability of milk and in the perceived qualities of cheeses.

Received September 27, 2006.
Accepted April 15, 2007.
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The introduction of several milk composition traits to the payment system for milk has not resulted in an appreciable change in these trends. Milk coagulation property (**MCP**) is an important characteristic and a basic requirement of milk for cheese production (Summer et al., 2002). The key milk properties for cheesemaking are good reactivity with rennet, a high degree of curd firming capacity, and good syneresis ability and whey expulsion. Commonly, MCP is measured as a combination of milk rennet clotting time (**RCT**, min) and curd firmness. Curd firming time is measured as the time required to achieve 20-mm firmness ($\mathbf{k_{20}}$, min), and curd firmness is measured at the end of test ($\mathbf{a_{30}}$, mm). These parameters describe the milk coagulation process: the enzymatic or primary phase after the chymosin effect, the nonenzymatic or secondary phase after the casein aggregation, and the last phase after the gel structure formation. The MCP is the determining factor in cheese quantity and quality and is highly variable. Studies comparing individual milk samples have shown that MCP is affected by physical and chemical parameters such as titratable acidity (Formaggioni et al., 2001), somatic cell count (Politis and Ng-Kwai-Hang, 1988), protein and casein contents, and calcium and phosphorus concentrations (Summer et al., 2002). Ikonen (2000) reported that milk which starts to aggregate soon after the addition of clotting enzyme and forms a firm curd within a reasonable time produces higher cheese yield. Several studies found a wide variation in MCP among different breeds (Macheboeuf et al., 1993; Mariani et al., 1997, 2002; Ikonen 2000; Malacarne et al., 2006) and among different cows in the same breed (Ikonen, 2000; Bittante et al., 2002; Tyrisevä et al., 2003). In addition, environmental factors play a fundamental role. Politis and Ng-Kwai-Hang (1988) reported the influence of DIM, age of cow, and month of sampling on MCP from individual milk samples. The objectives of this study were to investigate the effects of different breeds reared in the mountain region of Trento, Italy, on MCP and to examine the relationship between MCP and milk composition.

MATERIALS AND METHODS

Data Set and Collection of Milk Samples

Data for this study were composed of 506 herd milk samples from morning and evening milkings of the same day. Samples were collected with a milk yield meter, combined in a sample tube, stored in a refrigerator, and transported twice daily at about 4°C to the consortium of cooperatives of the dairy industry of the Trento province. Herd milk samples were collected at monthly intervals during 2001.

The structure of data analyzed is based on herds with only one breed (herd effect is nested within breed). Therefore, the breed effect was tested on the variance of the herd within breed effect. To estimate the breed effect, the planned sampling involved 55 random single breed herds located in the mountain area of the Trento province. The rearing conditions, in particular the feeding management, reflected the different maintenance requirements and production levels of these breeds. The structure data did not allow a complete distinction of breed and herd effects, and therefore the breed-estimated effect also includes a part of the rearing conditions effect.

The herds sampled included 8, 16, 10, 13, and 8 herds for the HF, BS, S, R, and AG breeds, respectively. The number of herd samples per breed were 83, 153, 86, 125, and 59 for HF, BS, S, R, and AG, respectively. Thus, on average, the numbers of samples per herd were 10, 10, 9, 10, and 7 for the HF, BS, S, R, and AG breed, respectively.

Cows calved during all seasons and the average lactation length (days) per breed was comparable: 176, 181, 135, 147, and 149 for the HF, BS, S, R, and AG breeds, respectively.

Laboratory Analyses

Analysis of MCP and other milk parameters (fat and protein percentages, somatic cell count, Soxhlet-Henkel acidity, and bacterial count) were performed by Concast laboratory (Trento, Italy). The MCP traits were analyzed without preservative within 6 h from collecting, and other qualitative analyses were carried out within 24 h on milk samples with Azidiol preservative (Concast, Trento, Italy). Chemical composition (fat and protein percentages) of milk was determined using Midinfrared instruments (IDF, 1995), somatic cell count was obtained using a direct microscopic method according to standard methods proposed by International Dairy Federation (IDF, 2000), and bacterial count was determined by Bactoscan 8000 according to standard methods proposed by International Dairy Federation (IDF, 1991).

The MCP measurements were carried out by Formagraph (Foss Electric, Hillerød, Denmark), which allowed the milk samples to coagulate for a maximum of 30 min (Annibaldi et al., 1977). Titratable acidity was determined according to the method proposed by Anonymous (1963).

Statistical Analyses

The ANOVA was defined to take into account that herd is nested within breed. Indeed significance of breed

Table 1. Least square means, standard errors, and significance of the breed effect (*P*) of milk composition traits per breed for herd milk samples

	Breed[1]											
	HF		BS		S		R		AG			
Trait	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	*P*-value	RMSE[2]
Protein, %	3.19	0.01	3.48	0.01	3.29	0.01	3.25	0.01	3.38	0.02	<0.001	0.106
Fat, %	3.59	0.03	3.82	0.02	3.82	0.02	3.39	0.02	3.72	0.03	<0.001	0.195
SCS, points	4.01	0.09	4.03	0.06	3.34	0.08	4.27	0.07	3.46	0.10	0.042	0.659
Titratable acidity, °SH/50 mL[3]	3.42	0.02	3.56	0.02	3.47	0.02	3.41	0.02	3.47	0.03	<0.001	0.180
LBC,[4] points	4.08	0.12	4.11	0.08	4.19	0.11	4.29	0.09	4.72	0.13	0.714	0.864

[1]HF = Holstein-Friesian; BS = Brown Swiss; S = Simmental; R = Rendena; AG = Alpine Gray.

[2]RMSE = root mean square error.

[3]°SH = Soxhlet-Henkel degree.

[4]LBC = log bacterial count.

effect was tested on the error line of the herd within breed variance as suggested by Snedecor (1967).

For statistical analysis, 2 models were used. In the first model, milk composition traits were analyzed as continuous traits using the GLM procedure of SAS (1996) according to the following linear model:

$$Y_{ijklm} = \mu + breed_i + herd_j(breed_i) + lact_k + season_l + e_{ijklm},$$

where Y_{ijklm} = protein and fat percentages, SCS, titratable acidity, log bacterial count; μ = overall mean; $breed_i$ = fixed effect of breed i (i = BS, HF, AG, S, R); $herd_j(breed_i)$ = fixed effect of the *j*th herd (*j* = 1...55) within *i*th breed; $lact_k$ = fixed effect of the *k*th herd average level of DIM (*k* = 1...5); $season_l$ = fixed effect of the *l*th bimonths of sampling (*l* = 1...6); e_{ijklm} = residual random error term ~ N (0, σ_e^2).

The season effects were classified in six 2-mo classes (1 = January to February; 2 = March to April; 3 = May to June; 4 = July to August; 5 = September to October; 6 = November to December); the herd average of DIM was categorized into 5 classes (1: <120 d; 2: 120 to 145 d; 3: 145 to 175 d; 4: 175 to 200; 5: >200 d).

To examine the milk characteristics on MCP, the RCT, k_{20}, and a_{30} traits were the dependent variables, and the second model considered the same effects in the first model along with titratable acidity, protein and fat percentages, SCS, and log bacterial count classified in 3 classes, low (x < mean – 1SD), medium (mean – 1SD ≤ x ≤ mean + 1SD), and high (x > mean + 1SD), within each breed. The level of significance was set to $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows least square means and significance levels of breed effects for composition traits in the herd milk samples. There were consistent differences ($P < 0.001$) among breeds for protein and fat percentage, SCS, and titratable acidity. The BS breed had the best milk quality characteristics with the highest percentage of protein (3.48%) and fat (3.82%), and titratable acidity (3.56 °SH/50 mL, where °SH is degrees Soxhlet-Henkel). The S breed had the lowest value of SCS (3.34) in accordance with a previous report by Vicario (2005), whereas the R breed had the highest SCS (4.27). Fat percentage and SCS of HF were similar to those reported for the same breed by Formaggioni et al. (2001), whereas the value of titratable acidity was similar to that reported by Summer et al. (2002).

There were 106 samples that coagulated with a value of a_{30} lower than 20 mm. Of these samples 49, 24, 20, 5, and 8 belong to HF, BS, S, R, and AG breeds, respectively. It was possible to define K_{20} values only for 400 of the 506 herd milk samples (79% of total data). The HF breed had the highest percentage of samples with poor rennet coagulation properties (55%) followed by S (23%), whereas R had the lowest percentage (4%). Statistical analysis of RCT and a_{30} used the total data set of 506 records, whereas the analysis of k_{20} used only 400 records with defined values.

Table 2 shows the variation sources of MCP traits. The coefficients of determination were 0.49, 0.39, and 0.32, respectively, for RCT, a_{30}, and k_{20}. Breed effect, tested against herd within breed, was the most significant source of variation for all MCP traits (RCT, a_{30}, and k_{20}). The RCT trait was significantly influenced by herd, DIM, month of sampling, and titratable acidity. Protein, fat, log bacterial count, and SCS classes were not statistically significant sources of variation for MCP traits.

The least square means of milk coagulation traits were, on average, better in the R breed than in the others breeds with 13.5 min, 27.0 mm, and 5.9 min for RCT, a_{30}, and k_{20}, respectively (Table 3). There were

Table 2. Significance of model effects for ANOVA of 3 milk coagulation traits for herd milk samples

Effect	df	RCT F	RCT P-value	k_{20} F	k_{20} P-value	a_{30} F	a_{30} P-value
R^2		0.49		0.32		0.39	
RMSE[2]		2.53		2.30		5.82	
Breed[3]	4	31.13	<0.001	5.83	<0.001	27.29	<0.001
Herd (within breed)	50	2.16	<0.001	1.28	0.108	1.43	0.036
Days in milk	4	5.13	<0.001	0.82	0.514	1.27	0.280
Month of sampling	5	8.99	<0.001	3.41	0.005	4.69	<0.001
Protein (within breed)	2	0.71	0.494	2.38	0.095	2.76	0.064
Fat (within breed)	2	1.06	0.347	0.05	0.954	0.12	0.891
SCS (within breed)	2	0.53	0.592	1.26	0.284	1.50	0.224
Titratable acidity (within breed)	2	8.08	<0.001	3.63	0.028	3.07	0.047
LBC[4] (within breed)	2	1.08	0.339	0.21	0.810	0.36	0.701

Trait[1]

[1]Milk coagulation traits: RCT = rennet coagulation time (min); k_{20} = curd-firming time (min); a_{30} = curd firmness (mm).

[2]RMSE = root mean square error.

[3]Tested on herd (within breed) variance.

[4]LBC = log of bacterial count.

no differences for RCT values among BS, S, and AG. The HF had the worst MCP values of 18.0, 17.5, and 8.2 for RCT, a_{30}, and k_{20}, respectively. As reported by several authors (Macheboeuf et al., 1993; Mariani et al., 1997, 2002; Ikonen, 2000; Tyrisevä et al., 2002), breed was an important source of variation for MCP traits. The values of MCP traits were similar to those reported by several authors. In particular, those of the BS were consistent with values reported in literature for individual milk samples (Mariani et al., 1997). Concerning the HF breed, the RCT and a_{30} values were similar to those reported by Summer et al. (1999) with the exception of k_{20}, which was lower in this study. The HF breed had the worst coagulation properties as reported by Macheboeuf et al. (1993) and Mariani et al. (2002) in the Parmigiano-Reggiano cheese production. The current study provides evidence that milk of the R breed began to aggregate 4.5 min earlier and curd was 9.5 mm firmer than milk of HF. It is important to emphasize that these are the first results regarding the MCP traits in the R and AG breeds. The desirable MCP traits and the low to medium protein content of the R milk appeared to be in contrast with the scientific literature where a positive but weak relationship between high values of MCP and high protein content has been reported (Ikonen, 2000). Several other factors influence MCP including casein content (Van Hooydonk and Walstra, 1987; Caron et al., 1997), κ-casein variants (Mariani et al., 1976, Okigbo et al., 1985; Davoli et al., 1990; Ikonen et al., 1997; Tyrisevä et al., 2003, 2004), β-LG genotypes (Kübarsepp et al., 2005), titratable acidity (Summer et al., 2002), pH (Lindström et al., 1984; Ikonen et al., 2004), and calcium content (Tervala et al., 1985; Kübarsepp et al., 2005).

The RCT values were better at earlier DIM, with 14.7 and 15.6 min for classes 1 (<120 d) and 2 (120 to 165 d), respectively (data not shown). These results agree with those reported by Davoli et al. (1990) and Okigbo

Table 3. Least square means and standard errors of milk coagulation traits per breed

Trait[1]	HF LSM	HF SE	BS LSM	BS SE	S LSM	S SE	R LSM	R SE	AG LSM	AG SE
RCT	18.0^{A}	0.4	16.1^{B}	0.4	16.2^{B}	0.4	13.5^{C}	0.4	16.0^{B}	0.5
k_{20}	8.2^{B}	0.6	6.4^{A}	0.4	7.1^{A}	0.4	5.9^{A}	0.4	7.6^{A}	0.5
a_{30}	17.5^{D}	1.0	24.1^{B}	0.8	21.9^{C}	0.9	27.0^{A}	0.9	21.2^{C}	1.1

Breed[2]

A–DLetter differences in rows are significant at $P < 0.01$.

[1]Milk coagulation traits: RCT = rennet coagulation time (min); k_{20} = curd-firming time (min); a_{30} = curd firmness (mm).

[2]HF = Holstein-Friesian; BS = Brown Swiss; S = Simmental; R = Rendena; AG = Alpine Gray.

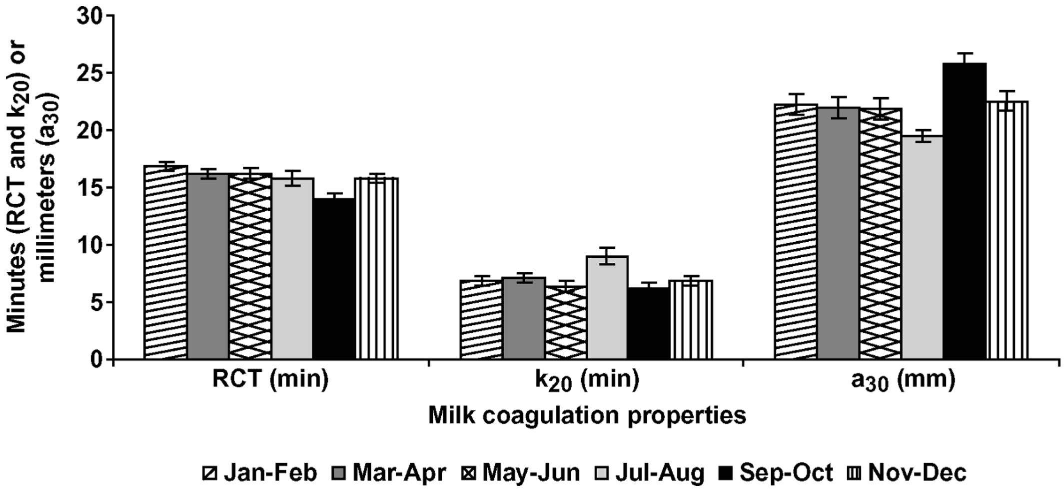

Figure 1. Least square means (with SE whiskers) of effect of months of sampling class on milk coagulation traits. RCT = rennet coagulation time (min); k_{20} = curd-firming time (min); a_{30} = curd firmness (mm).

et al. (1985). The RCT values increased with stage of lactation; milk from the first 120 d of lactation began to aggregate 2.4 min earlier compared with milk obtained in late lactation (>200 d). These results match those reported by Davoli et al. (1990) and Tyrisevä et al. (2004). In other research, MCP were at their best from 5 to 30 DIM, and again after 240 DIM (Kreuzer et al., 1996; Ikonen et al., 1997; Ostersen et al., 1997; Kübarsepp et al., 2003; Tyrisevü et al., 2003). However, according to Lindström et al. (1984) and Pagnacco and Caroli (1987), DIM had no effect on the MCP.

The RCT and a_{30} values were on average better in September and October samples (14.3 and 25.7 mm, respectively; Figure 1). The worst values of a_{30} and k_{20} occurred during warm periods when an unexpected climatic condition was experienced, as reported by Piazza (2006), confirming the results of a study conducted in 1998 on HF by Summer et al. (1999). The seasonal variations in MCP traits are probably due to the effect of high temperature and humidity values on metabolic-nutritional status of the cows (Summer et al., 1999).

Titratable acidity plays an important role in all phases of milk rennet coagulation and depends on individual and herd effects. Reactivity between rennet and casein, aggregation rate of paracasein micelles, and syneresis ability of the curd (Summer et al., 2002) are influenced by titratable acidity, and this represents an important parameter for the technical evaluation of the dairy-technological quality of milk. Low acid levels in milk manifested considerably longer RCT and k_{20}. In fact, 30 min after rennet addition, such milk supplied a curd with very low firmness if compared with that of milk with normal titratable acidity. In fact, different RCT (min: 15.2 class 3, 16.2 class 2, and 17.2 class 1), a_{30} (mm 23.8 class 3, 22.5 class 2, and 20.8 class 1), and k_{20} (min 6.2 class 3, 6.9 class 2, and 7.7 class 1) were registered (Figure 2). These results and the variation of MCP traits were similar to those reported in other studies concerning titratable acidity in the HF breed (Okigbo et al., 1985; Macheboeuf et al., 1993; Formaggioni et al., 2001).

CONCLUSIONS

This study has shown that MCP is highly variable, especially in relation to cow breed and sampling season. Milk of the most important dairy breed, HF, had a fair aptitude milk quality for cheese making. The other breeds produced milk characterized by a better MCP than HF. A small-sized local breed of the Alps, R, had the best results, confirming that the risk of extinction of some breeds, and the consequent reduction of biodiversity, can cause a loss of economically valuable genetic characteristics and that this risk is not yet fully known and understood.

The positive milk coagulation ability of R milk seems not to be due to its protein content or other quality characteristics analyzed, as in the case of BS, but to other aspects that still have to be investigated. Further research on these local breeds could lead to a better understanding of the mechanisms involved in the cheese-making ability of milk. Moreover, the local breeds are often characterized by robustness, fertility, longevity, and adaptability to the mountain environment that, at least in part, can compensate for their lower level of milk production. However, their gap in production, and thus in revenues, compared with HF is increasing, and their risk of extinction is growing.

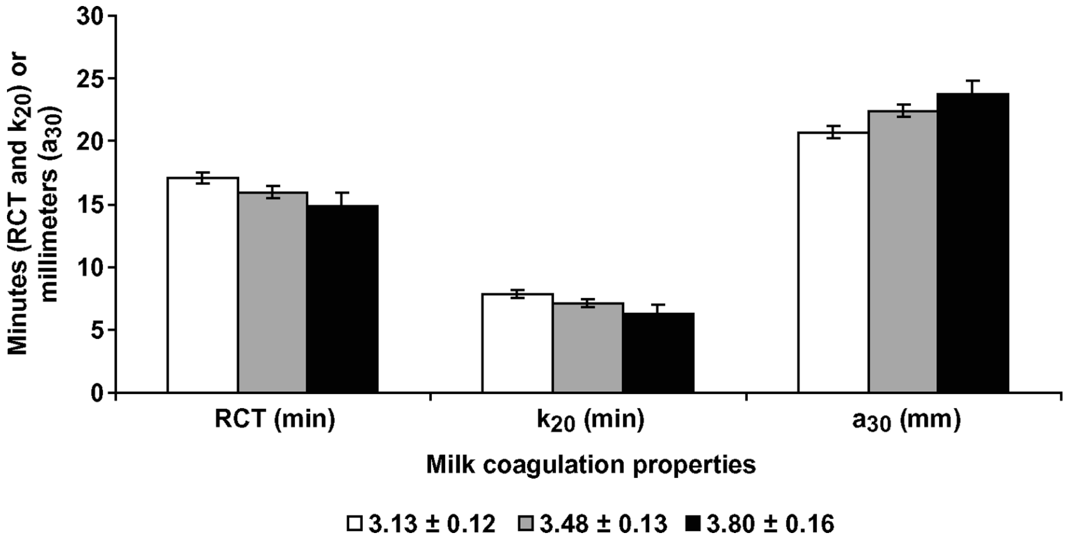

Figure 2. Least square means (with SE whiskers) of effect of titratable acidity classes on milk coagulation traits. RCT = rennet coagulation time (min); k_{20} = curd-firming time (min); a_{30} = curd firmness (mm).

The development of a payment system for milk that takes into account the intrinsic value for cheese-making ability could be an important opportunity for the conservation of these endangered genetic resources. Beyond their genetic value, these breeds exert a positive influence on sustainability of milk production in fragile environments, like mountain areas. They also preserve an important cultural value (history, traditions, arts, and literature).

ACKNOWLEDGMENTS

Authors want to thank Consorzio Trentingrana – Concast (Trento, Italy) and Superbrown Consortium of Bolzano and Trento for providing data. The authors also thank PRIN 2005 (2005074889_002) for partial financial support and Sarah Loker for her suggestions.

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